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THE ANALYSIS OF THE ORGANISMAL DIFFERENTIALS OF GRAY NORWAY RATS AND OF TWO MUTANT RACES BY MEANS OF TRANSPLANTATION[1]

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TRANSPLANTATION of different kinds of tissues carried out under experimentally varied conditions led to the concept of the organismal (individuality, species or class) differentials, chemical characteristics which are present in the large majority or all of the tissues of an individual, species, genus or class and differentiate these from other individuals of the same species or other species, genera or classes.[2] The nature of the organismal differentials depends upon the genetic constitution of individuals and species or larger groups, and further analysis has shown that multiple factors determine the character of the individuality differential. These genetic factors lead to the production of substances which, when introduced into organisms possessing different organismal differentials, cause incompatibilities between host and transplant. Such incompatibilities manifest themselves in two ways. In the first place, certain tissues of the host (lymphocytes, connective tissue and blood vessels) react against the transplant in a graded manner, the reaction being the stronger, the greater the differences between the organismal differ-

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[2] Leo Loeb, *Biological Bulletin*, 40: 143, 1921; *Physiol. Review*, 10: 547, 1930.

entials of host and transplant, and secondly, the transplant is injured by the body fluids of the host. Furthermore, the strange organismal differentials alone or in combination with other substances may serve as antigens which call forth production of immune substances directed against the transplant. The intensity of these reactions corresponds to the degrees of differences in the character of the organismal differentials and therefore also in the genetic constitutions of host and transplant. In general, the constitution of the organismal differentials of two individuals corresponds to their family relationship; but because of the existence of individual variations between organisms possessing the same family relationship, such as brothers, it is necessary to take the averages of a number of individuals representing certain relationships in order to make this correspondence evident. The series of genetic constitutions on which the character of the organismal differentials depends and the corresponding series of averages of relationships correspond therefore to the series of reaction-intensities on the part of the tissues and body fluids of the host against the graft. And each of these series can be represented by a spectrum, that of the genetic constitutions and of the averages of relationships and that of the reactions. Through close inbreeding it is possible gradually to transform a homoiogenous relationship into a syngenesious one and ultimately to make it closely approach the autogenous condition, which characterizes different tissues belonging to the same individual.

While the genetic constitution is the factor ultimately determining the kind and intensity of the reaction between host and transplant, there are in addition other secondary factors which are important, such as the age of the donor of the transplant and of the host. In young animals the reactions are on the average less severe and the transplant correspondingly remains better preserved than in older animals.

In former investigations various parts of the spectrum

of relationship have been analyzed by means of transplantation. We reported also several years ago on a limited number of transplantations between ordinary white rats and cream and hooded rats, obtained from the Wistar Institute. However, it was of interest to analyze further by means of transplantation the changes in relationship which are caused by mutations. Mutations which appeared in a strain of Gray Norway rats, studied since 1919 by Dr. Helen Dean King at the Wistar Institute, seemed to offer material suitable for this purpose.[3] During the period of fifteen years six different heritable mutations have appeared in these Norway rats; they all affected the hair. For our work we used (1) the offspring of the original Gray Norway rats; (2) Albino mutants which appeared first in the fourteenth generation of the Gray Norways bred in captivity at the Wistar Institute. Fifteen Norway females in the thirteenth to the twenty-first generation cast among their offspring 91 Albino rats, representing almost 10 per cent. of the total offspring; about nine tenths of the offspring were Gray Norways. As far as body growth and weight of organs is concerned these Albino rats have the characteristics of Wild Norways. They carry genetic factors both for self (uniform coat color) and gray; (3) the second mutation which we used, "Curly Coat," first appeared in the seventeenth generation of the Gray Norways. This is a dominant mutation affecting hair structure; at certain periods in the life of these rats their body hairs all show a twist or kink. This mutation differs from the smooth coat of the original Gray Norways by a single genetic factor.

As in the majority of our previous investigations we used for transplantations as a rule one lobe of thyroid gland and a piece of xiphoid cartilage to which in some instances a piece of bone was adjoined. Besides cartilage and thyroid tissue, these transplants often contained also parathyroid, fat tissue and striated muscle, as well as

[3] Helen Dean King, *Proc. Sixth Internat. Congress of Genetics*, 2: 108, 1932.

bone and bone marrow. We were thus able to judge the effect of the new host on a variety of transplanted tissues, which differed much in degree of sensitiveness to injurious factors. In every case the tissues were transferred into pockets in the subcutaneous tissue of the host. The grafts were removed for microscopic study in the majority of cases after 9, 12, 16 or 20 days. The thyroid transplants were cut into complete serial sections, while sections were made through various parts of the cartilage graft. In this way we studied: (1) 45 syngenesiotransplants in Gray Norway, Mutant Albino and Curly Coat rats. (2) 86 pieces of homoiotransplants from Gray Norway to Gray Norway, from Mutant Albino to Mutant Albino, and from Curly Coat to Curly Coat. The animals used in these experiments were not brothers and sisters or parents and children, but in some cases they may have been more distantly related. (3) 102 pieces of intermutant transplants were grafted. Transplantations from Gray Norway to Mutant Albino, from Albino to Curly Coat, from Gray Norway to Curly Coat and the reciprocal transplantations served for this purpose. (4) From Gray Norway, Mutant Albino and Curly Coat, 116 pieces were transplanted to tame white and hooded rats; the corresponding reciprocal transplantations were also made. (5) In addition, one autotransplantation was carried out in a Gray Norway rat. Not included in this summary are those transplantations in which the transplants were not recovered at the conclusion of the experiment. Altogether 351 transplants were thus studied microscopically.

In order to summarize and to compare the results obtained in the different series of transplantations we used two methods. In the first place, we adopted a system of grades indicating the intensities of reaction of the host against the transplant; these intensities were averaged in the various series. Secondly, we selected certain characteristic features, relating to the mode of reaction of the host tissues against the graft and to the state of preserva-

tion of the transplant, and we compared the frequency with which certain results were obtained in the different series of transplantation. Thus we compared for instance the various degrees of preservation of the thyroid and parathyroid. We also compared the frequency of complete or partial preservation of cartilage and perichondrium, the occurrence of partial or extensive necrosis in these tissues. In the case of the fat tissue we used the intensity of invasion and replacement of this tissue by connective tissue and also the occurrence of necrosis as tests. In addition we determined the intensity of lymphocytic infiltration in the various transplanted tissues. We furthermore noted the frequency of the occurrence of regenerative processes in the cartilage and striated muscle tissue, as well as of the replacement of bone marrow by connective tissue in the bone transplants. On the whole, the conclusions at which we arrived by means of these two methods were concordant. We shall therefore state only the results obtained by grading the transplants as a whole in the different series.

Grades of Transplants. The thyroid and cartilage transplants were graded in the following way:

Grade 6. Corresponds to the typical results of autotransplantation. Thyroid represents an oval, the outer part of which consists of well-preserved acini containing solid colloid and mostly closely adjoining each other; in other cases thin strands of connective tissue may separate some acini. In the center, the connective tissue is on the whole loose in structure. There may be present some ducts or pearls composed of squamous epithelium. Cartilage, perichondrium and fat tissue are well preserved, although as a result of accidental injury during the process of transplantation there may be small areas of necrosis in cartilage and perichondrium and perhaps new formation of cartilage. Muscle fibers are well preserved and at the end of the cut fibers some regenerative processes may occur with the formation of nuclear chains. The bone marrow is more or less preserved, but the bone cells for the most part degenerate.

Grade 5. Corresponds to results obtained in a favorable syngenesiotransplantation. The thyroid transplant differs from Grade 6, in that a more marked lymphocytic infiltration begins in places inside or outside of the ring of thyroid acini; the lymphocytes begin to penetrate between some acini. In certain areas there may be a slight increase of fibrous tissue between the acini leading to some defects in the thyroid ring. In the cartilage transplants the preservation of the tissues is still excellent on the whole, but there may be some beginning ingrowth of connective tissue into the fat tissue separating some peripheral fat cells or there may be seen collections of lymphocytes in the fat tissue.

Grade 4. Instead of an oval with loose connective tissue in the center, we find a more or less round mass of denser fibrous tissue, in the peripheral parts of which there is a ring of mostly well-preserved acini. The ring is not as wide nor as complete as in Grade 5. In a considerable number of acini the colloid may be lost. The amount of fibrous tissue separating or replacing the acini in certain places is greater and there occur variable and even considerable accumulations of lymphocytes in the transplant which may destroy some acini. A slight destruction of acini by lymphocytes and connective tissue may take place. In the cartilage grafts there may or may not be some necrosis of cartilage and perichondrium. While the greater part of fat tissue has been well preserved, there is a somewhat more extensive ingrowth of connective tissue between the fat cells and in spots foci of fibrous tissue may replace fat tissue. The amount of lymphocytic infiltration in the fat tissue and in places along the perichondrium and bone is variable. Although the muscle tissue is no longer especially well preserved, regenerating muscle fibers are often seen. The bone marrow is beginning to be replaced by ingrowing fibrillar connective tissue, but some large capillaries and a few bone marrow or fat cells may still be preserved. When cartilage is necrotic, or when it has been injured, regenerative new formation of cartilage may take place.

Grade 3. The condition of the thyroid resembles Grade 4; but the ring of acini is much more incomplete here. A considerable part of the peripheral ring of acini has been replaced by fibrous tissue, in other places connective tissue may grow between new acini. Fibrous bands may surround and separate bundles of acini. Variable degrees of lymphocytic infiltrations are found and lymphocytes and connective tissue cells may penetrate into and destroy some acini. In the cartilage graft some parts of cartilage and perichondrium and perhaps small portions of the adjoining fat tissue may be necrotic; also small areas of hemorrhage, in various stages of absorption and organization, are sometimes seen between the fat cells. One half or perhaps even a little more of the fat tissue is often replaced by fibrous tissue and lymphocytes. Lymphocytes may also accumulate in places around cartilage and bone. The fat cells frequently are separated by somewhat larger cells taking up small fat droplets. Some fat cells may coalesce, or at least the fat, formerly contained in several adjoining fat cells, may flow together into one large drop which may be surrounded by some connective tissue. Regeneration of necrotic or injured cartilage may occur. There may also be seen some regenerating muscle fibers; but these regenerative processes are very restricted in extent. The bone marrow is usually partly necrotic and more or less replaced by fibrillar connective tissue.

Grade 2. The thyroid is largely replaced by fibrous tissue and by lymphocytes, but some small collections of acini are often preserved in the periphery of the transplant; usually these are embedded in dense fibrous tissue and surrounded or invaded by some lymphocytes. Especially at early stages following transplantation, some remnants of necrotic and hemorrhagic thyroid tissue are usually found in the center of the transplant. In the cartilage specimen there may be considerable amounts of necrotic cartilage, perichondrium and fat tissue. Hemorrhages and fibers of fibrin in process of organization

may be seen in the latter. While certain areas of fat tissues are still preserved, the greater part of it has been replaced by fibrous tissue more or less infiltrated with lymphocytes. Regeneration of cartilage may still occur at points of necrosis or injury, but regenerating muscle tissue is no longer seen. The bone marrow is either necrotic or replaced by fibrillar connective tissue, with or without masses of lymphocytes.

Grade 1. The thyroid has been entirely destroyed or replaced by fibrous tissue infiltrated with variable numbers of lymphocytes. Cartilage and perichondrium show differing degrees of necrosis. Some regeneration of cartilage may still be seen occasionally or necrotic parts may be infiltrated with connective tissue cells and lymphocytes. The latter process may also be found in transplants which received Grades 2 and 3 and in which necrosis of parts of cartilage and perichondrium has taken place. The fat tissue is almost entirely replaced by fibrous tissue containing variable accumulations of lymphocytes, although some hemorrhagic or necrotic areas in process of organization may occur in the transplanted fat and connective tissue. Otherwise the state of the transplants resembles that seen in Grade 2.

In general the transplants can be graded fairly accurately in this way. This applies especially to the thyroid transplants. Here the grading is more simple and accurate than in the case of the cartilage, where a greater number of factors, some of them subject to the influence of accidental conditions, has to be considered. Repeated grading of the same specimens of thyroid gland at different times led to grades which were almost identical. In the grading of cartilage the results are somewhat less concordant, although in the large majority of cases the agreement obtained in repeated gradings is close enough to inspire confidence in the classification of the results thus arrived at. If discrepancies occur between the gradings of thyroid and cartilage, more importance should be attached to those of the former tis-

sues. The transplants taken out for examination at different times following the operations show considerable differences; the greater the number of transplants, the more trustworthy are the results; therefore, the grades given to the specimens examined 12 and 16 days following transplantation can claim the greatest consideration, while the number of specimens examined after 20 days is usually much smaller and their grades can, therefore, be considered only as of confirmatory significance. The grades given to the cartilage transplants represent the means between two independent gradings. In general, if the transplant is very much injured by the body fluids and tissues of the host, the connective tissue invasion predominates; while, if there is less injury, the transplant and host being more suited to each other, although a certain degree of incompatibility still exists, the lymphocytic infiltration tends to predominate. The grades

TABLE I

SUMMARY OF AVERAGE GRADES GIVEN TO TRANSPLANTS OF THYROID, CARTILAGE AND ADJOINING TISSUES IN THE VARIOUS SERIES OF EXPERIMENTS

I. Autotranspl.	20	days.	Thyroid.	1	Gray Norway	rat:	6			
II. Syngenesiotranspl.	12	days.	Thyroid.	6	rats:	4.7;	Cart.	6	rats:	4.15
in Gray Norway	16	"	"	9	"	3.4	"	10	"	3.2
and Mutant rats.	20	"	"	7	"	2.0	"	7	"	2.1
III. Homoiotranspl. in	9	"	"	6	"	3.0	"	6	"	2.7
Gray Norway	12	"	"	11	"	3.0	"	11	"	3.3
and Mutant rats.	16	"	"	14	"	2.6	"	15	"	2.5
	20	"	"	13	"	2.4	"	11	"	2.0
IV. Interracial trans-	9	"	"	7	"	3.1	"	6	"	2.6
plantations be-	12	"	"	18	"	2.6	"	17	"	2.6
tween Gray Nor-	16	"	"	18	"	2.0	"	18	"	2.3
way and Mutant	20	"	"	9	"	1.6	"	9	"	2.0
rats.										
V. Transplantations	9	"	"	8	"	2.5	"	8	"	2.3
from Gray Nor-	12	"	"	25	"	2.0	"	25	"	2.6
way and Mutant	16	"	"	20	"	1.5	"	20	"	2.5
rats to tame Al-	20	"	"	5	"	1.7	"	6	"	2.6
bino and Hooded										
rats and recipro-										
cal transpl.										

obtained in the different series of transplantation are given in Table I.

DISCUSSION

From a study of Table I, the following conclusions may be drawn: (1) Autotransplantation of the thyroid gland leads, in the wild rats, to about the same perfect results as in other strains or species of animals tested so far.

(2) In series II, syngenesiotransplantations mostly from brother to brother, the average results of transplantation of thyroid as well as of cartilage were better than in series III, IV and V. We notice furthermore that with increasing length of time during which the grafts were kept in the host the results deteriorated. Even in closely related animals therefore the relative incompatibility which exists between graft and transplant leads to a gradual injury with increasing length of time.

In series III, homoiotransplantations, the grades are lower than in syngenesiotransplantations, if corresponding periods of time in both series are compared. However, after nine days the tissues may not yet have recovered entirely from the injury inflicted on them through the process of transplantation and the grades may therefore be somewhat lower at nine than at twelve days.

This applies in the case of the cartilage transplantations also in series IV, where the average cartilage grade was the same at nine and at twelve days and in series V, where it was even better at twelve than at nine days. But thyroid transplants were always better preserved at nine days after transplantation than at later periods and, in general, there is noticeable throughout the different series a decrease in preservation of the transplant and an increase in the injurious effects exerted by the host on the transplant with increasing time. In series IV, interracial transplantations, there is apparent a further decrease in the degree of preservation and an

increase in the severity of the reaction, as compared with the findings in series II and III.

In series V, in which tissues from Gray Norway and Mutant rats were transplanted to tame Albino and Hooded rats and *vice versa,* the same conclusion holds as far as the thyroid transplants are concerned; the results are less favorable, as compared with those obtained in series IV, and an increasing deterioration takes place from the ninth day to the twentieth day following transplantation, except that the grade at twenty days is slightly better than at sixteen days; this is presumably an accidental effect to be accounted for by the relatively small number of transplants at the twenty-day period. In the case of the cartilage transplants, the grades at twelve and sixteen days are similar to the corresponding ones in series IV.

If, without separating thyroid and cartilage transplants, we compare the average grades of thyroid and cartilage at twelve and sixteen days, at which periods the number of experiments is largest, we obtain the following figures: Series II: 3.9, Series III: 2.9, Series IV: 2.4, and Series V: 2.2. In Series I, the grade would have been 6. If we compare the corresponding figures for thyroid transplants at 12 and 16 day periods in the different series, we obtain the following figures: Series I: 6, Series II: 4, Series III: 2.8, Series IV: 2.3, Series V: 1.8. These figures indicate the increasing deterioration of the transplants with increasing distance of relationships. At the same time we notice that the more unfavorable the results of transplantations become, with increasing distance of relationships, the smaller is the difference in the grades between the successive series. This is due to the fact that, if once a certain distance of relationship has been reached, the outcome is so unfavorable that a still greater distance can not cause a much greater deterioration.

If we compare (Table II) the averages of the highest grades of the thyroid transplants in the different series,

TABLE II

HIGHEST AND LOWEST INDIVIDUAL GRADES

		Thyroid	High	Low	Av. High	Av. Low	Cartilage	High	Low	Av. High	Av. Low
I. Autotransplantation			6								
II. Syngenesiotransplantation	12 days	"	5.5	3.4			"	4.7	3.4		
	16 "	"	5.2	2.3	4.8	2.2	"	5.6	1.8	4.7	2.2
	20 "	"	3.6	1			"	3.9	1.5		
III. Homoiotransplantation	9 "	"	3.9	2			"	3.9	2.1		
	12 "	"	5.5	1			"	5.5	1.9		
					4.7	1				4.3	1.7
	16 "	"	4.8	1			"	4.4	1.6		
	20 "	"	3.9	1			"	3	1.5		
IV. Interracial transplantation	9 "	"	3.8	1.6			"	3.1	2.3		
	12 "	"	4.3	1			"	3.9	1		
					3.9	1				3.6	1.3
	16 "	"	3.5	1			"	3.9	1.4		
	20 "	"	3.8	1			"	2.9	1.4		
V. From wild rats to tame Albino and Hooded rats and reciprocal transplantation	9 "	"	3.5	1			"	3.1	1.5		
	12 "	"	4.2	1			"	4.5	1.3		
					3.4	1				3.6	1.5
	16 "	"	3	1			"	3.1	1.7		
	20 "	"	3	1			"	3.2	1.5		

we notice that they are best in Series I and II and decrease gradually with decreasing relationship. In the averages of the periods from twelve to twenty days, the highest grades are: Series I: 6; Series II: 4.8; Series III: 4.7; Series IV: 3.9; Series V: 3.4. The averages of the lowest grades are: Series I: 6; Series II: 2.2; Series III: 1; Series IV: 1; Series V: 1. In the case of the cartilage transplants, the averages of the highest grades are: Series I: 6; Series II: 4.7; Series III: 4.3; Series IV: 3.6; and Series V: 3.6. The corresponding averages of the lowest grades are: Series I: 6; Series II: 2.2; Series III: 1.7; Series IV: 1.3; Series V: 1.5.

If, instead of comparing the averages, we compare the individual grades in the various groups, we notice that they are to some extent overlapping, the same grades occurring in different groups; yet the highest grades are higher in syngenesio- and homoiotransplantations than in interracial transplantation, and moreover in syngenesiotransplantation the higher grades occur more often than in homoiotransplantation. With increasing distance of relationship, the amount of necrosis in the transplants and the amount of substitution of the necrotic tissues by connective tissue become greater. The lymphocytic infiltration is the more marked, the greater the distance of relationship between host and graft, provided the difference between the organismal differentials of host and transplant is not so great that it leads to extensive necrosis of the transplant and largely to the replacement of the necrotic tissue by fibrous tissue. Such a graded replacement by connective tissue and also by lymphocytes can be followed especially clearly in the transplanted fat tissue; on the whole, the lymphocytic infiltration is more marked around and in the thyroid than in the cartilage transplant. It is also of interest and in accordance with what should be expected that, as a rule, the reactions in the two transplanted tissues, thyroid and cartilage with their associated tissues, in the same host agree with each other; thus for example, if in the thyroid transplant the lymphocytic reaction is strong, it is usually marked also in the cartilage transplant.

The reactions in interracial transplantations in rats vary therefore in individual cases in intensity; they still fall within that part of the spectrum of reactions which characterizes non-related members within the same race (homoiogenous relationship), but within the homoiogenous range of the spectrum they are situated at the end furthest from the point representing autogenous relationship. In individual cases there is a considerable variation in the intensity of the reactions, although the best grades here do not equal the highest reached in

homoiotransplantations. There is, however, no sharp break between the character of homoiotransplantations and of interracial transplantations, such as is found if we pass from homoiogenous or interracial transplantations to transplantations between nearly related mammalian species.

CONCLUSION

We may conclude from the results of these transplantations that the part of the spectrum of relationship, corresponding to racial differences originating by mutation, overlaps with the homoiogenous area of the reaction spectrum, but it is shifted towards the distant end of the latter area. There is therefore no sharp demarcation between the ordinary homoiogenous relationship and the interracial relationship, as far as our experiments may serve as an indicator of such a relationship. However, while we consider this conclusion as in all probability correct, we must recognize that two complicating factors enter into these experiments. In the first place, the age of the animals used in different experiments varied and we know that the intensity of reactions in transplantation is affected by this factor. Secondly, the litters of rats used for homoiotransplantations were frequently related to each other. This second factor may possibly have mitigated somewhat the severity of the reactions in homoiotransplantation, although not to a large extent. Notwithstanding the presence of these variable factors, it seems probable that the differences in the severity of reactions, which we observed between homoiogenous and interracial transplantations, is a real one. In favor of this interpretation we may also cite our former results, in which we carried out transplantations between different races of rats and in which we found a greater severity of the reaction in interracial than in homoiogenous transplantations. It is therefore probable that a single mutation, such as caused the change from "Gray Norway" to "Curly Coat," may have a definite effect on the organismal differential.

GENE RELATIONSHIPS IN TWO SPECIES OF MICE WITH REFERENCE TO THEIR POSSIBLE EVOLUTIONARY SIGNIFICANCE

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A STUDY of the genetic constitutions of two related species, when interfertile, is of interest from the standpoint of genetics since the inheritance of their specific characteristics can then be investigated. Such a study is likewise of interest from the standpoint of the evolutionary origin of the species, furnishing, as it does, a rough measure of their distance from a common origin and information concerning the development of the wild-type complex.

In plants a considerable amount of work has been done along these lines. The interspecific crosses within the genus Gossypium reported by Harland (1933) and the wider intergeneric wheat-couch grass hybrids by Verushkine and Shechurdine (1933) are among the many which have thrown light on the perplexing problems attending hybridization. Among animals, especially among mammals, species crosses are less commonly fully fertile, although sub-species have been utilized successfully. The work of Sumner and his associates (1932 *et ante*), later continued by Dice (1933), on the sub-species of Peromyscus are probably the most extensive investigations in this field.

As Fisher (1928) has pointed out, the substitution of a gene by its allelomorph is the only genetic mechanism of evolutionary significance known. This being the case, then genes for homologous characters in two related species can exist in any or all of four conditions: (1) On different chromosomes; (2) as allelomorphs; (3) same basic gene with different modifiers; (4) identical.

The first situation, in which genes for homologous characters lie on different chromosomes in the two species, seems to occur seldom within a genus, although it exists in different related genera. This condition could arise through independent mutations or through the fragmentation of the chromosome after the mutation had originally occurred. The presence on one chromosome of the genes for albinism and brown coat color in rabbits but not in mice is a case in point and may indicate the great period of time elapsing since the separation of the two forms.

The second condition, in which the genes of one species are largely allelomorphs of those of its relative, is well exemplified by *Gossypium hirsutum* and *G. barbadense,* as reported by Harland. This should indicate that, although the two species are closely enough related to be interfertile, they are farther apart than two forms in which more genes are identical.

When two species have, for the most part, the same basic genes but with different sets of modifiers conditioning the expression of the gene, we probably have the situation operating in the majority of fertile mammalian species crosses. Such crosses yield information concerning the nature of dominance.

With genes in related forms identical, we have the situation prevailing within domesticated races of a species, although even there probably some different modifiers may be at work. At least it seems reasonable to assume that a closer relationship is indicated than in any of the three classes above.

If two species can be crossed with fertile offspring resulting, thus permitting the production of back-cross and F_2 generations when necessary, information can be obtained regarding the relative fixity of the basic dominant complex of the ancestral protospecies before divergence into the two forms. Among mammals few cases of complete fertility of interspecific hybrids have been demonstrated. Although Sumner worked almost en-

tirely with subspecific crosses in Peromyscus he obtained one F_1 interspecific hybrid which was successfully back-crossed to representatives of both parental species. Dice, likewise, has obtained some species hybrids in the same genus. After all, it seems probable, according to the present-day taxonomic conception of the problem, that species and sub-species differ for the most part only in degree of constant differences and that, from an evolutionary standpoint, a sub-species may be merely an incipient species.

For some years the Jackson Laboratory has had a stock of the Chinese mouse, *Mus bactrianus,* identified as apparently intermediate between the sub-species *gansuensis* and *tantillus* (Allen, 1927), on the whole probably nearer the former. They differ from *Mus musculus* in a number of morphological details, among which are fewer caudal vertebrae, smaller size and differences in proportion of bodily and cranial parts. Later, Hicks and Little (1931) determined serological differences between representatives of these two species. There seems never to have been any question among taxonomists as to the specific distinction of these two forms of mice.

Mus bactrianus is a sandy-backed, white-bellied murid resembling in color the ordinary white-bellied house mouse, with the dorsal surface, however, visibly brighter. Genetically, they can be classified as intense, black, white-bellied agouti animals (DBA^w), although, as will be brought out later, modifying factors influence the expression of some of these genes. In the laboratory, they have been crossed with domesticated strains of *Mus musculus* carrying members of some dozen allelomorphic pairs of genes.

The white-bellied agouti gene (A^w) in *bactrianus* appears to differ somewhat from that found in the house mouse. In fact, its expression varies even within the race of *Mus bactrianus.* While in some, the belly hairs are white to the base, in others, they are faintly gray-based; rarely the whole ventral surface has a slightly

grayish cast. *Mus musculus* animals carrying the gene A^w, on the contrary, seem always to have ventral hairs with darker bases. The dorsal surface, as previously stated, also differs from agouti *musculus,* probably because of the wider yellow bands on the agouti hairs.

In crosses with non-agouti strains of *musculus,* the hybrids, although agouti, differ noticeably in color from the *bactrianus* parent. The belly is less often pure white, usually darker and often of a nearly uniform grayish or buffy hue. The dorsal surface likewise is rarely as bright, being generally much darker. Apparently, the width of the sub-apical yellow band on agouti hairs, as well as the number of interspersed black hairs, is dependent on auxiliary genes, and not fully determined by the gene A^w. To use another terminology, this gene, in so far as it determines belly color, is not completely dominant over its allelomorph lower in the series. The two species possess different modifying factors affecting the expression of A^w, if indeed the gene is identical in the two species.

In back-cross and F_2 generations, a wide range of dorsal and ventral coloration is found, the former ranging from that in animals phenotypically indistinguishable from *bactrianus* to others so dark as to appear almost black. The ventral surface varies from pure white to a uniform grayish condition. This segregation again suggests the presence of multiple genes, influencing the expression of the white-bellied agouti gene, which are not the same in the two species.

The gene for black (B) seems to be identical in the two forms, both in its effect on pigmentation and in its apparently complete dominance over the *musculus* recessive allelomorph, brown (b). If modifying genes are present, their effects on the main gene are not visibly different in the two species.

In considering intense (D) and dilute (d) pigmentation, the gene for the former as found in *bactrianus* seems to be the same as found in *musculus* with a similar

dominance over dilution. Probably in the hybrid generations (back-cross and F_2) a somewhat greater variation in the expression of the gene is present than is the case with the preceding pair of allelomorphs. Since dilution is brought about by the clumping of pigment granules, it seems logical to expect that a greater range in variability might occur than in the characters differing in color of pigment.

The Chinese species is dark-eyed (P) and dominant over the pink-eyed mutation (p) which has arisen in the house mouse. Extracted pink-eyed hybrids do not differ noticeably from extracted recessives from an intraspecific *musculus* cross.

A like situation is manifested in crosses between *bactrianus* with the gene for color (C) and albino *musculus* with the recessive gene (c). The former is as fully dominant as the *musculus* gene C, with the hybrid albinos also appearing the same. *Bactrianus* has not been mated to *musculus* carrying others in this series of multiple allelomorphs.

The smaller species in common with wild house mice carries the gene for normal ears (Se). Again this appears to be the same gene in the two forms, in both completely dominant to short-ear (se). If supplementary factors have any influence on the relative ear length of extracted hybrid recessives, it is too slight to be apparent. It is true, however, that but few measurements have been taken on this point; detectable differences might be distinguished if careful measurements were taken on a sufficient number of animals.

In the case of a new *musculus* recessive, leaden (l), the dominant condition, non-leaden (L), is found in *bactrianus* as well as in the wild house mouse. Non-leaden is similarly effective in both species with no apparent differences. Since this mutation is so recent a departure from normality, it is not strange that no distinct modifiers influencing its expression have arisen in the one species or the other.

Individuals of *Mus bactrianus* are "self"-colored, *i.e.*, they do not possess the gene for recessive spotting (s). Often, within the species *musculus* a recessive spotted strain, crossed with a self strain, produces F_1 animals showing a small forehead blaze. In fact, in many such crosses, a blaze or a belly spot is almost characteristic of a heterozygous piebald. When self *bactrianus,* on the contrary, was mated to piebald (white face) *musculus* only one F_1 individual out of a large number displayed a blaze and that consisted of merely a few white hairs. In the F_2 generation a great variation was shown in the extent of unpigmented areas, ranging from a small blaze to a much higher degree of piebald than was present in the *musculus* race. Since in nearly 500 animals the ratio of self to piebald showed a 9:7 ratio, it seems probable that the *musculus* white-face pattern is dependent on two recessive genes acting in conjunction. Judging from the F_1 hybrids, however, it appears that the *bactrianus* S gene, with its accompanying dominant complex, suppresses the expression of s more successfully than does S in *musculus.*

When *bactrianus* was mated to a race of Japanese waltzing mice (which according to Gates (1926) are probably derivatives of *Mus bactrianus*) with a high grade of piebald, the F_1's were white-bellied black agouti self animals. Those individuals, however, whose ventral surfaces had a grayish hue, seemed without exception to have an area of clearer white in that region. This may have been due to a heterozygous condition of the spotting gene, although this appears improbable, since in the F_2 and subsequent generations such a condition was universal, or nearly so, among the non-agouti segregates as well as among those agoutis whose bellies were not pure white. Investigations on spotting are still in progress and will be reported later in a more extended form.

Japanese waltzing mice, in addition to a high grade of piebald, are homozygous for the recessive character

"waltzing," (v), in which an inability to continue long in a straight line with resulting rotation is manifested, due to the structure of the internal ear. The F_1 animals (*bactrianus* × waltzing) are normal and in the F_2 generation waltzing and normal segregate in the expected numbers exactly as they do in *musculus*-waltzing crosses. It seems that the gene for normal (V) is identical in the two species.

The recessive genes of the nine allelomorphic pairs thus far described agree in that they have all—with the possible exception of waltzing—arisen in *Mus musculus* and, with the few minor differences noted, behave alike to the wild type as found in both species. Wright (1929) has pointed out that mutations most frequently represent inactivations of genes and that, for simple physiological reasons, inactivation should generally behave as recessive. This perhaps explains why the group of recessive *musculus* genes are equally recessive to the wild type dominant complex of the smaller congener, even though a distinct species.

In addition, however, *bactrianus* has been mated to four *musculus* strains carrying genes dominant to wild type: the so-called dominant lethals, yellow (A^y), brachyury (T) and dominant spotting (W), and the not necessarily lethal, naked (N). These genes should give a clearer picture of the differences in reaction of the wild types in the two species than the recessive genes. If wild type is to be considered as the optimum—or at least, successful—normal balance attained through the process of evolution, the effect of a wild type complex with its numerous modifying genes on the dominant characters in the hybrids should be farther in the direction of normality, the farther that particular complex has diverged from the wild type which gave rise to the dominant mutation. In other words, the dominant gene must be of such a nature in its relation to the wild type gene complex from which it originated that its expression is permitted away from normality. When introduced into another wild type complex not identical from an evolutionary

standpoint, its deviation may be lessened and in the direction of the normal condition.

The gene for yellow, A^y, is a member of the agouti series of multiple allelomorphs, dominant to A^w, A, a^t and a, and lethal when homozygous. When *bactrianus* was mated to yellow *musculus,* both yellow and non-yellow were produced according to expectation. The only visible difference in coloration was that, in general, the yellow hybrids were brighter yellow, less sooty, than yellow *musculus.* The presence of the non-agouti gene (a) in the latter but not in the former may account for the difference since Keeler (1931) has stated that A^ya mice are often sooty yellow. At most, such differences as were present were slight, so it may be stated that as far as color is concerned, the A^y gene behaves similarly towards *bactrianus* and *musculus.*

As is well known (Danforth, 1927), the A^y gene has, besides its influence on color, a tendency to produce excess adiposity. This abnormal adiposity is almost as characteristic of the gene as the yellow color. Within the species *musculus,* yellow animals are much heavier and fatter than their non-yellow litter mates. The situation regarding growth in yellow and non-yellow *bactrianus-musculus* hybrids is now being investigated. Although no mice have as yet reached advanced ages, the indications are that the difference between yellow and non-yellow hybrids is not as great as that in the two classes of *musculus.* At an early age, 91 days, yellow hybrids seem to be about 18 per cent. heavier than their non-yellow sibs, while in animals produced by mating yellow *musculus* to an unrelated strain of the same species the corresponding figure is roughly 30 per cent.

The dominant gene for brachyury (T) has the effect of reducing tail length. Dobrovolskaia-Zavadskaia (1933) has suggested a series of symbols for representing phenotypic types, with the symbol T for the basic gene. Short-tailed animals within the strain vary from complete taillessness to those with tails more than half normal length. When crossed with an unrelated *mus-*

culus strain the tails averaged a little longer but when mated to *bactrianus* were almost as long as normal tailed (tt). Chesley (unpublished) reported similar findings for *musculus-bactrianus* hybrids. This recalls Fisher's theory of dominance in which, in brief, dominance may be due not to the gene itself but to the action of modifying genes. An unrelated strain of the same species might be expected to carry fewer modifying factors permitting dominance of T than the brachyury strain, while a different species would carry still fewer. Such are the results for which Fisher's theory supplies a plausible, although by no means demonstrated, explanation.

The remaining dominant *musculus* genes which have been introduced into *bactrianus* hybrids are naked (N) and dominant spotting (W) with but few animals produced in either case. The former, not quite in the same category as the other three dominants, apparently has slight if any differential effect, judging merely from inspection, on the two species. The latter gene, independent of genes for recessive spotting, manifested itself characteristically by producing white feet. Since *bactrianus* and its hybrids normally have unpigmented feet, it was impossible to distinguish those animals with the W gene from those without it. No dorsal spotting whatever was present in any of the hybrids.

Besides characters conditioned by a single gene, *bactrianus* has been crossed with *musculus* strains differing in characters dependent on multiple factors. Among such are number of caudal vertebrae, size of body as a whole and of separate parts, proportions of skull, etc. Some of these have been reported on in earlier papers (Green, 1931, 1932a, 1933, 1934). These characters, however, are less useful in a study of comparative gene relationships than are characters due, loosely speaking, to a single gene.

Gates (1926), working with a cross between a strain of Japanese waltzing mice, of which the ancestral stock was presumably *Mus bactrianus,* reported F_2 and backcross data which he interpreted as showing the formation

of "association systems" of chromosomes, since the sets of characters derived from the respective parent races tended to remain together. The writer (1932) using data derived from wild type *Mus bactrianus* with the genes D, B and A^w, and *Mus musculus* with the corresponding recessive allelomorphs d, b and a, found no such association but only random assortment, although nearly 1,600 back-cross and F_2 mice were involved. Since then several hundred more have been obtained with the same situation still prevailing. It thus appears that, as far as these three allelomorphic pairs are concerned, Mendelian behavior is identical in both intra- and interspecific crosses.

SUMMARY

The results obtained in crossing *Mus bactrianus* with strains of *Mus musculus* carrying a number of recessive gene mutations indicate that, in general, the wild type complex is similar in the two species. The basic genes investigated are, for the most part, probably the same, but with their expression influenced by different modifiers. With certain dominant *musculus* gene mutations the situation differs somewhat. Although dominant over the wild type *bactrianus,* the expression in the hybrids is much lessened and in the direction of the normal condition found in both forms. The wild type complex found in a species is probably the balanced result of an evolutionary process and, since recessives seem most frequently to represent inactivations of genes, for physiological reasons is similarly dominant over the recessive genes of another species. On the other hand, the expression of a dominant gene, which must bear such a relation to the wild type complex from which it originated that its expression is permitted away from normality, is as a result often reduced toward the normal condition when introduced into a second species with a somewhat different complex. The balance in mice was probably attained very early, and in consequence the ancestral species was

essentially stable before divergence into the closely related forms *Mus bactrianus* and *Mus musculus*.

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ASPECTS OF THE RELATION BETWEEN GENES AND DEVELOPMENT IN DROSOPHILA[1]

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THE relation of genes to characters includes all the processes of development, from the gene in the chromosome to the end result. It is worth while first to consider what are the essential and what are the secondary characteristics of the problem.

If we were to list what information is desirable, it would be, first of all, knowledge of the relation of the gene to the active product in the cytoplasm; of the arrangements in the cytoplasm for using the active product; of the possibilities for genic interaction within the nucleus, and afterwards, for interactions of the products in the cytoplasm; and of a number of other such properties of the system. All these are essentials of the problem of the effects of genes on development. We should subsequently turn our interest to the secondary interactions which occupy the embryologist, the relation of genes to organizers, and so on. Finally we should study the actual appearance in development of the characters differentiating, let us say, one mutant race from another.

From this point of view, it is clear that the essentials are precisely the problems which are least accessible. The reason is simple: if we knew enough about development, and about cellular physiology in general, it would be possible to use the effects of genes on development to find out something about the nature of the gene. Contrariwise, were we familiar with the properties of the

[1] Part of a symposium on "Genes in Relation to Characters," held at the joint meeting of the Genetics Society of America and the Western Society of Naturalists, at Berkeley, California, on June 20, 1934. Much of the work reviewed in this talk was undertaken during tenure of a National Research fellowship in zoology, at the Laboratory of Biophysics, Columbia University, New York, 1927–29.

gene from independent evidence, we might proceed to unravel the peculiarities of the developmental system. But our problem is, knowing neither, to solve both at once.

Those who are at work on *Drosophila* find a peculiar point to the question. For the genetic material available is all that could be desired; and even embryologically, experiments can be done, as Geigy (1932) and Howland and Child (1932) and Howland and Robertson (1934) have demonstrated. It is for us to make use of these opportunities; we have a complete story to unravel, because we can work things from both ends at once. What I propose doing here is to review some of the ways of studying the problem that are available, and in particular those with which I myself am familiar. Taken by and large, they constitute an attack on the analysis of the developmental processes which genes affect. Thus they can touch only the periphery of the really important problems, yet still seem necessary preludes to the real advances.

Manifold Effects and Developmental Processes

It is a familiar idea that no characteristic of an organism is exempt from the effects of gene changes, and in fact this seems to be the case in *Drosophila*. The numerous mutations discovered affect all conceivable characters; yet different genes may produce, when they change, mutant races which are indistinguishable from each other. This peculiarity would seem at once to indicate that the genes may be divided into groups, according to their major effects—a division which is of course automatic in planning experiments. But genes have not only major, but also minor, manifold effects, studied particularly by Dobzhansky (1927, 1930). If we are to picture them as cooperating in groups to effect specific processes, we must be clear as to what the problem of manifold effects involves.

The very fact that a given gene affects two apparently unrelated developmental processes may mean either that an unsuspected relation exists between the two processes,

or that the gene is capable of exerting more than one primary effect. There is a possible way of deciding between these alternatives, which is not worked out as yet, but may be mentioned. We may examine the genes having certain given effects, and see whether or not they are likely to have similar manifold effects. In other words, is there a correlation between an effect on one organ and that on another, in an array of mutant genes? I have made a tabulation of what descriptive data there are on the mutants of *Drosophila,* and it appears indeed that for certain effects correlations do exist. For example, 7 per cent. of the genes examined affect eye texture and size but not wing venation; 5 per cent. affect the wing venation but not the eye. However, as much as 7 per cent. affect both eye texture and wing venation—an apparent correlation. The data are not as yet good enough to warrant more than a tentative conclusion, but we can test this in another way.

It is well known that the results of extreme environmental shocks during development often simulate genetic effects. Such abnormalities are, for example, found subsequent to treatment with high temperature, as Goldschmidt (1929) has found in *Drosophila,* and particularly Kuhn (1932) in *Ephestia.* Now if the same treatment is given at different times during development, different effects are produced; and by examining the manifold effects in these cases, the problem may be restated in terms of time. For example, I kept Drosophila larvae for twelve hours at 37° C. Those treated when five days old gave 26.6 per cent. flies showing both bristle and wing modifications, but when the treatment was given at six days, only 10.4 per cent. belonged to this group. On the other hand, among the five-day-old larvae, 17.9 per cent. showed modifications of the wings only, but in the six-day-old group this had increased to 38.5 per cent. It is already evident that experiments such as these give some ground for the belief that some manifold effects may be related to the time of occurrence of given

developmental processes. More data are needed before the two types of manifold effect—induced and hereditary—can be compared; and it is only by means of such comparison that the problem is solvable. It is even possible that it may provide a method for studying what has become something to conjure with—the time of action of genes.

Analysis of a Single Developmental Process

The study of the manifold effects of genes may permit their classification into groups according to the developmental processes which they affect. But the processes themselves must be studied by detailed analysis of selected systems, which present definite advantages for work. I shall not now consider the analyses that have been made in a number of cases, but discuss in a little detail one with which I myself have been concerned—that of the eye colors of *Drosophila* (Schultz (1929, 1932)).

The advantages of pigments in studies of this sort have been too often stated to need repetition here. To these the *Drosophila* eye colors add the variety of genes available for interaction studies, the ease with which the developmental history may be followed and the clearness of the histological pattern.

The normal wild type eye of *Drosophila,* first studied histologically by Johannsen (1924), contains in its pigment cells deep red granules, orange granules and yellow granules. The histology of the eye has since been studied in more detail by Hertweck (1932). For the present purposes we may concern ourselves with the occurrence of pigment in three places—in the primary and secondary cells and beneath the basement membrane. The red and the orange granules occur in both the primary and the secondary cells. They constitute most of the pigment in the wild type eye. What yellow granules there are, occur in the primary cells.

Now, in the pupal development of the fly, a series of changes occurs, as the pigment granules are formed. In the early pupa, the eye is transparent, but at 25° C.,

about fifty hours after pupation, the first signs of color appear around the outside of the eye. The invasion continues until, about seventy-five hours after pupation, the eye is completely tan, or brown, in color. Histologically, it appears that the first pigment is formed around the basal membrane, then in the secondary cells and finally in the outer primary cells. Then, at about eighty hours after pupation, a color change occurs; the tan becomes red and the definitive eye color is obvious. But, like the original formation of pigment, this color change is also centrifugal; for the primary cells in the adult eye still retain yellow granules.

What are these pigments and what is their relation to each other? They may be extracted separately from the wild type eye by the use of solubility differences and of differences in their absorption spectra. The absorption spectrum of a water extract from a wild type *Drosophila*

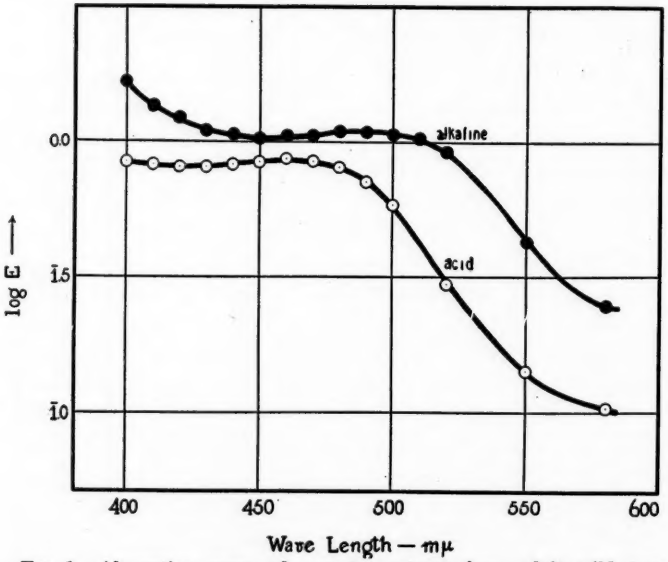

FIG. 1. Absorption curve of a water extract from adult wild type *Drosophilas*. The abscissae are the wave-lengths of light, the ordinates the logarithms of the relative extinction coefficients.

is shown in Fig. 1. The abscissae are the wave-lengths of light, the ordinates the logarithms of the relative extinction coefficient, a quantity proportional at any given wave-length to the concentration of pigment. In acid solution the curve is flat until λ 480, when it sinks gradually. In alkaline solution a shift occurs to λ 500—the pigment is a hydrogen ion indicator. On analysis, this curve is seen to be a composite. The red and yellow granules actually correspond to red and yellow pigments, which are closely related, but can be separated by means of their solubility differences.

Fig. 2 shows the absorption curve of the red pigment,

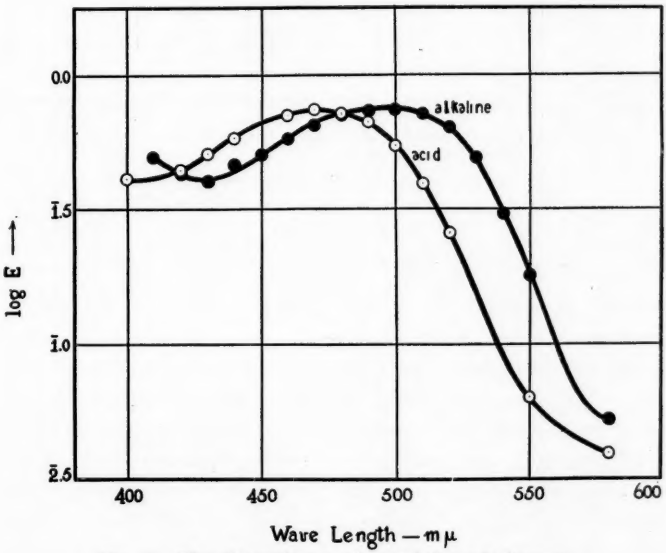

FIG. 2. The absorption spectrum of the red pigment.

which clearly must have been the main component in the whole extract. Its absorption maximum is at λ 470 in acid solution, where it is yellow; and in alkaline solution, where the pigment is red, there is a shift to λ 500.

The yellow pigment presents a different picture (Fig. 3). Its curve is much steeper, and in accordance with the

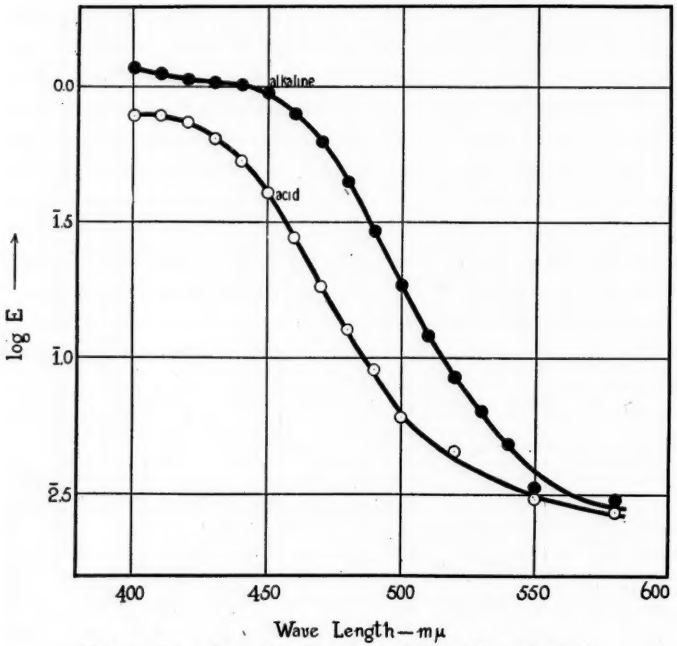

FIG. 3. The absorption spectrum of the yellow pigment.

yellow green color of the solution, the maximum is towards the violet at λ 430.

The pigments are water soluble, but do not dissolve in any of the usual organic solvents. They form esters, are of a low molecular size, passing through membranes which hold Congo Red; they form insoluble salts with silver and mercury, and are easily oxidized to colorless forms by H_2O_2 and similar oxidizing agents. No reversible oxidation reduction reactions have so far been found in vitro. Both pigments, the red and the yellow, may by certain treatments be changed into a third pigment, whose absorption curve is shown in Fig. 4.

In spite of the failure to obtain reversible oxidations in vitro, in smears of the eye, an oxidation may be brought about from red to yellow by the use of H_2O_2, an oxidation which may be reversed by the use of sodium

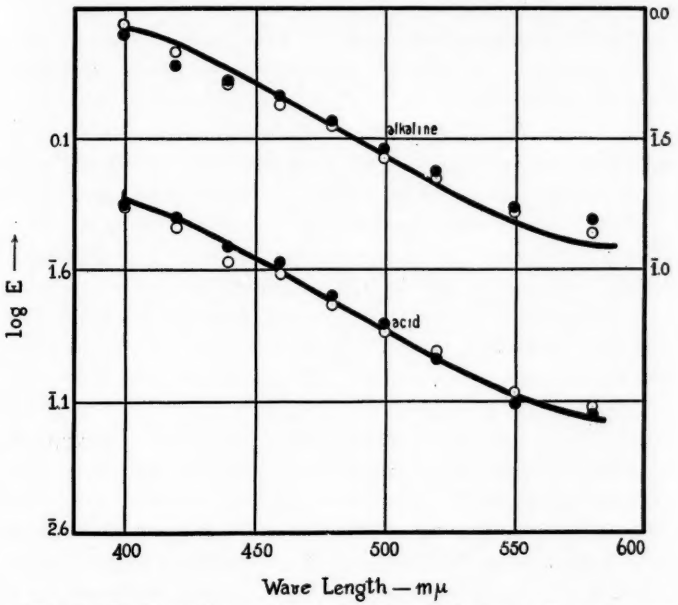

FIG. 4. The absorption spectrum of the brown pigment. The same curve has been fitted to the data from acid and alkaline solutions. There is a slight change in the shape of the curve on passing from acid to alkaline pH, but the major change is in the amount of light absorbed.

hydrosulfite and which is not of an enzymic nature. This reversible reaction immediately prompts the question whether the color changes in development are of the same nature. Is the change from yellow to red a reduction? By experiments on the pupae, treated at proper stages with gaseous H_2S, it is found that indeed the tan of the early stages can immediately be changed to red. Even more, if a light enough treatment is given so that the pupa continues development, the red color changes back to tan, subsequently to return to red at its normal time. It seems probable from these experiments that the red and yellow pigments are so simply related to each other as oxidation-reduction products. The failure to obtain this in vitro is probably due either to technical difficulties or to an effect concerned with the nature of the

granule containing the pigment. On such a basis, the problem of pigment synthesis is enormously simplified. There are not two independent pigments, but only one to deal with.

If then we consider the normal development of pigment in the *Drosophila* eye, it becomes intelligible in terms of the synthesis of a pigment according to a definite pattern, and a subsequent change in its state of oxidation, also according to a pattern. What now of the mutant races? Are they part of the same scheme?

The contrast between different groups of eye color mutants has been realized in *Drosophila* from the very beginning, since the early experiments of Morgan and Bridges (1913). Some are bright red, others are pinkish; still others verge into the brown shades. This rough classification becomes clearer when the histological picture is examined.

In one group are to be placed the bright red eye colors. These have red or orange granules and no yellow ones. They have less pigment below the basal membrane, and dense aggregates in both primary and secondary cells.

In another group may be placed those which have the opposite kind of picture: few red granules or none at all in the primary cells. In the secondary cells they have either deep red and yellow granules, or yellow granules only.

Still another group is intermediate, containing the orange-red and the yellow granules, with the pattern characteristic of the first group. These groupings at present do a sort of rough justice to the facts. They are probably too few, and as we shall see presently, it is possible that each locus has its own group.

All the eye colors in these groups, despite the differences, give the same kinds of pigment as does the wild type. They differ in the relative amounts found; and in some cases, such as vermilion for the red and sepia for the yellow, only the one type of pigment is present. It is clear from this result that in all the mutant types of

Drosophila the fundamental plan of eye pigment formation is unaltered—the changes do not concern the nature of the pigment synthesized.

Within this grouping the widest variations obtain. Both the total amount of pigment and the concentrations of the pigments relative to each other are varied. Only a beginning has been made as yet in the quantitative analysis of pigment variation. Technical difficulties in the demonstration that the extracts really give a quantitative picture of what is present in the eye, remain to be overcome.

It is, however, the developmental history of the eye colors that gives, as one might expect, the key to the situation. Table 1 shows the percentages of development completed at the time when pigment first appears, and when the bristles are observed to darken, for a group of twenty-three eye color types. The latter criterion, darkening of bristles, coincides with the change of color from tan to red in the wild type, at 25° C.

It is clear that for the first group of eye colors the onset of pigment formation coincides roughly with that of the wild type. This happens with such relatively light eye colors as apricot or with dark ones like sepia. Next there is a group of mutants which are doubtfully the same as the preceding. Actually they are more different than the numbers show, since the later increase in concentration is visibly slower.

But the striking difference occurs with the scarlet-like eye colors. These show very little pigment until just before the time when the wild type changes from tan to red. At that time a pale yellow color appears, and shortly thereafter, it changes to orange, while the depth of color increases rapidly in a sudden gush.

These different groups of genes can be considered as the reflection of two aspects of eye color development, which may be called the onset and increase of pigment formation; or better the early and later phase, to avoid prejudicing the issue. It is evident from the behavior

TABLE 1

THE COLOR CHANGES IN THE PUPAL DEVELOPMENT OF THE DIFFERENT EYE COLOR TYPES OF DROSOPHILA

Type of eye color		Percentage of pupal development complete at	
		First appearance of color	Darkening of bristles
GROUP 1	apricot	53	81
	brown	53	83
	carnation	48	79
	clot	52	74
	prune[2]	49	76
	purple	51	71
	sepia	55	79
	wild type	55	83
GROUP 2	garnet	56	77
	light	57	82
	peach	55	74
	ruby	53	74
GROUP 3	cardinal	63	74
	cinnabar	71	77
	scarlet	68	77
	vermilion	71	75
Combinations—within Group 1			
	prune carnation	51	74
between groups 1 & 2			
	prune garnet	53	74
between groups 1 & 3			
	purple cinnabar	75	75
	vermilion carnation	70	70
	vermilion sepia	75	75
between groups 2 & 3			
	scarlet peach	76	76

of the mutant eye colors that these are relatively independent; the scarlet group, in which the onset of pigment is late, has its differential reaction from the wild type early, the garnet and brown groups later.

The final pigments are the same, as I have said. One might question the intermediates, however. Nevertheless, the sequence in the vermilion group, in which there is

only a transitory yellow stage, is homologous to that of the wild type. The yellow in the early vermilion stage is reduced to red by H_2S, completely comparable to the behavior of the tan stage of the wild type. But the smears of the adult eye show no oxidation-reduction reactions—a situation which has been shown to depend not on the color of the pigment but on some peculiarity of the adult vermilion eye. I suspect it is granule size, since I have found differences in granule size between sepia, wild type and the vermilion eyes—the sepia granule measuring .24 μ, the wild type and vermilion .19 μ, figures gained by measuring photographs under the microscope.

It appears then that all these different mutants find their places within this relatively simple scheme, involving modifications in degree of changes which take place in the normal fly. How far are these changes intra-cellular? This can be measured for some cases with a high degree of certainty, for others considerable caution is necessary. In most cases, the effect probably involves at most a small group of cells, since in mosaics even single ommatidia are distinguishable. In the case of vermilion eye (and one may guess the same holds for the other eye colors of the group) Sturtevant (1933) has shown that extra-cellular influences are involved, probably residing in the gonad; both gonad and eye must be of the same genetic constitution to permit the manifestation of the vermilion effect. This seems peculiarly relevant to the fact that the differential reaction between the wild type and mutant becomes apparent earlier for the vermilion group than for any of the others. The earlier such a reaction occurred, the more likely it would be to involve correlations between organs in later development.

It may be remarked that the eye color genes also have manifold effects of a sort, in that they determine the color of ocelli and of testis sheath. Here, too, the same grouping is possible. The color of the testis sheath appears later in development than does the eye color,

and it may hence be surmised that the persistence of the grouping is due to the similarity of processes involving synthesis and deposition of pigment in the two tissues.

These data on the relations of the mutants to the wild type make the differences between them intelligible in developmental terms. They do not in themselves give any information as to how, to use a familiar phrase, the gene acts. They are, however, essential if we are at all to try to work at the problem. Without them the attempts at inference from the end products have a certain scholastic flavor which results quite naturally from the involved argumentation which must intervene between each proposition and its demonstration. In the case of interaction effects, for the study of which all these data are a necessary preliminary, this is especially true.

The analysis of the behavior of genes in combination with each other forms a bridge between the description of development in the different mutant races—what may be called Mendelian embryology—and the study of gene action proper. The logic of the analyses is simple; will the effect of genes A and B in combination be the sum of their separate effects? If not, how far back must we go before we reach a condition where each effect is specific for the gene concerned, and independent of the others? If we can go back far enough, it is conceivable that such conditions will be found.

I have studied a large number of eye gene combinations qualitatively and some quantitatively. The salient feature of all such combinations in the adult has long since been recognized; they are usually at least as dilute in eye color as the lighter of the two types used. Studied in detail, the combinations corroborate the classifications of the eye colors made on the basis of the purely descriptive data.

If the groups correspond to something in the animal, differences should be found between combinations of eye colors within a group and those between groups. Combinations between mutants of different groups should

show the effects of two relatively independent reactions, and their differences from either single eye color should be marked. Within groups, on the other hand, the combination should be only slightly different from the lighter single type; the genes affect the same reaction, and the more extreme member of the combination should be epistatic.

In development, moreover, deposition of pigment should begin at the time characteristic of the earlier of the two components. At the later stages, the formation of pigment should be determined by the member of the group whose differential reactions occur at that time.

In effect this is the case; the pattern of the pigment distribution in a combination is governed by the earlier of the two groups, and the type of pigment produced, by the later. Thus the combination vermilion sepia has little pigment below the basal membrane, much in the primary cells—like vermilion; but the pigment produced is all yellow, like that of sepia. The nature of the granules, however, is like that of vermilion; no reduction of the pigment can be obtained, showing that the curious behavior of the vermilion group in this regard is definitely connected with some other factor than the formation of red rather than yellow pigment in the granule.

About a hundred such effects have been studied, all of which appear to conform to the rule. They confirm the idea that at least two relatively independent processes are concerned in eye color development. In addition, they offer at least a statement of the problem in terms of the genes concerned. The interactions as observed appear to be summation products of independent reactions. Each gene does its own job. But this is the object of the work; if it should be quantitatively verified, a great many things will become possible. The specific properties of each locus will then be accessible—a problem which can only be discussed to a certain extent at present.

ALLELOMORPHISM AND SPECIFICITY

The specificity with which each locus produces its effects may be seen from a consideration of heterozygotes, containing non-allelomorphic genes which produce similar effects. In the case of the eye colors this is quite striking; recessive mutants which are complete mimics of each other when crossed give perfectly normal-appearing heterozygotes. This is, of course, the usual test for the absence of allelomorphism; but the end effects may be extraordinarily similar.

This test deserves further consideration. It involves not only the relations of the two loci, but at each locus the relation of one allelomorph to the other. It is possible to conceive of an arrangement whereby no difference in specificity between the two loci is involved at all, but only a difference in relative amounts at each locus. At the present juncture this does not seem very profitable, particularly since the problem may be attacked more directly. It is the problem of dominance in another form, presenting both its aspects at once.

Dominance involves the reactions relative to each other of a pair of allelomorphs working in a specified environment, internal or external. For the moment, we may postpone theoretical considerations, but consider the problem only in its relation to the underlying question of specificity—the differentiation of genes from each other.

One possible method of attack is the attempt to lower the threshold and observe what then occurs in the double heterozygote. This is of particular interest in cases like that of the eye colors, where there is information as regards the developmental system and interaction effects of the genes concerned.

Cases of so-called conditioned dominance have long been known in the eye colors, where genes, ordinarily recessive, show dominance in the homozygote for some other eye color mutant. It is interesting to consider whether this phenomenon shows any relation to the grouping of eye colors which has just been discussed.

Out of thirty-seven cases examined, the five cases of conditioned dominance found showed no specific relations between groups. This is obviously too small a number to permit any conclusions, but I think a systematic study of the much larger numbers possible should show whether the conditioning of dominance involves a specific type of reaction between the conditioner and the gene being studied, or whether a general group reaction is involved.

The study of the eye colors from a direct developmental point of view leads then to the consideration of what the possibilities of action are within the set of genes itself. This is not unexpected, since the work was undertaken with that end in view, because in *Drosophila* we are able to handle the genic end of the reaction so easily.

Quantitative Effects and Genic Balance

The study of interaction effects finds a powerful aid in the analysis of the effects of quantitative differences at a given locus. Here, instead of studying what happens when one gene is substituted for another, we deal with simple alterations in amount.

The first and classical example of such studies is that of Bridges (1922) on sex, shown to be quantitatively determined in *Drosophila* by the relation between numbers of X and autosomes. More recently, Dobzhansky and I (1934) have been able to show that in the X itself the effect is a quantitative one, determined by many factors. But I shall not consider this case in detail now.

We have available in *Drosophila* a body of descriptions of character changes resulting from duplication or deficiency for a wide variety of chromosome regions. This provides a method which, when combined with the direct developmental study, should give valuable information about the relations of genes to each other. By the use of this double attack, it is possible to vary the amount of genic material at a locus, and consider directly its effect on the developmental process.

There is very little as yet to say about such a synthesis;

most of the work has been concerned with either the one aspect or the other. The earliest studies of such effects, by Bridges (1917) and by Mohr (1923), were concerned with the manifestations of recessive genes included in deficiencies. In certain such cases, it was found that the recessives show in a much more extreme form than usual. This is the so-called exaggeration effect, most simply explained as the result of one gene's not being able to do the work of two. The contrary type of case was first studied by Sturtevant. He was able to show that in the case of the Bar gene the observed differences in end effect were due to the presence of one, two, three or four Bar genes. Zeleny and his students, in the meantime, have concerned themselves with the analysis of the development of the eye facets in the Bar types, both histologically, and by the use of differences in temperature sensitivity at different periods. Hersh in particular has analyzed the data from such experiments, making it intelligible in terms of general concepts about growth. But there has been as yet no simultaneous consideration of the two methods of attack in this case.

Similarly, the work of Stern (1929) and later of Muller (1933) on the effects of addition of genes at a given locus has concerned itself only with end effects. Stern found that by increasing the number of mutant genes at the locus of bobbed, keeping the genic balance aside from that locus constant, he got a steady increase in bristle length, which approached the wild type asymptotically.

Muller, with League and Offerman (1932, 1933), has approached the problem in the same way by using extra fragments containing mutant genes. In several cases they found that an addition of such genes produced an effect closer to the wild type. The conclusion was, as in the case of Stern, that these genes were doing the same thing as their normal allelomorphs, only doing it less effectively. In certain other cases extra fragments produced either no effect at all or an intensification of the mutant effect. Here they judged that the mutant was doing

something different from the wild type, either something entirely new or else antagonistic to the normal allelomorph.

In none of these cases has there as yet been an attempt to provide a tangible developmental interpretation. I do not propose to give such a case now—there is none. But it may be interesting to consider whether the grouping of eye color mutants which I have discussed has any relation to the results from such dosage experiments. There are, all told, some nine cases at present in which dosage changes have been observed for eye color loci. They involve all three groups, but there is no detectable correlation at present between the type of effect obtained by adding or subtracting an allelomorph, and the group to which such a gene belongs. It is possible that this result and the specificity which was discussed in the cases of conditioned dominance are related. These two ways of analysis, it would seem, present the simplest method available of bridging the gap between the embryological and genetical information.

I should like now to consider more closely the general problem of genic balance. It was from the beginning evident, as the early Mendelian discussions attest, that the unitary genes must somehow interact in the developing organism. We have already considered, in the case of the eye colors, the method of genic combination for studying this problem; and pointed out earlier the possibility of using manifold effects and direct embryological experiment for another attack. We have just considered how the quantitative changes at a given locus may be used. All these methods involve changing one factor and keeping the rest constant in so far as one can. But it is possible that the thresholds are determined, in our experiments, by the factors kept constant, and when these are changed, a rather different picture may obtain. The simplest changes are of course the quantitative ones involved in the addition of a polyploid set. These have already shown their importance both in Bridges' experi-

ments and in those of Dobzhansky and myself on sex. The latter are particularly instructive, since when a change was made in threshold it appeared that factors, apparently ineffective under the usual conditions, in reality were quite effective.

A rather striking case of the sort can also be found in the behavior of the white allelomorphs. These have been used by Muller, League and Offerman in their experiments, and have shown an increase in depth of color with an increase in number of genes. They differ, however, among themselves as regards their behavior in male and female, some being very much lighter in the male, others darker. This difference can be shown, by appropriate experiments with fragments of the X chromosome outside the white locus, to depend upon the relations of genes within the X. But this is not all. Let us consider the behavior of these allelomorphs in the triploid, with 3 X's and 3 sets of autosomes, and compare that with their behavior in the intersex, which has only two X's and still has three sets of autosomes. It seems first that the triploid and diploid females have identical eye color. Secondly, the intersexes show for each type of allelomorph a difference from the triploid similar in sign to that between male and female. Thirdly, and most important, these differences are intensified in the intersex. It should be noted that the ratio differences between male and female are greater than those between triploid and intersex, and yet the eye-color difference between the two latter is much more striking. It is evident that before it is possible to accept the conclusions from dosage experiments in the diploid, these experiments must be checked by the triploid.

This is easy to do in these cases, by the use of a deficiency; thus we may compare the effect of two doses in both triploid and diploid, everything else being constant. It appears indeed that the effect of two doses is less than that of three, and hence the difference between triploid and intersex is due to the dosage relations of some factor

in the X outside the white locus. But it also follows from this result that the requirements from that locus are greater in the triploid than in the diploid, since the effect of three doses in the triploid is equivalent to that of two in the diploid.

A similar result I have found more recently in studies of the fourth chromosome character shaven. The fourth chromosome presents especial advantages for this type of work, since flies containing as few as one or as many as four members of this group can be obtained. Table 2 contains a brief summary of the manifestation of the shaven gene in different doses in the diploid and triploid. In the diploid, two doses of shaven are sufficient to remove the abdominal and, occasionally, some thoracic bristles. The same number of fourth chromosomes in the triploid is as extreme as the very much stronger allelomorph of shaven appropriately called naked. Let us add another fourth chromosome containing shaven, the diploid becomes nearly wild type, the triploid is—as it should be, since now the balance is the same—like the diplo-shaven diploid. I have in addition seen the diploid haplo-IV shaven, which is more extreme than the diplo-shaven triploid; the tetra-shaven triploid, which is practically wild type; and probably the tetra-shaven diploid which has many extra bristles. The evidence becomes complete with the consideration of the heterozygotes. Shaven is completely recessive in the diplo-IV diploid; but in the diplo-IV triploid it is dominant, giving a phenotype similar to that of the triplo-shaven diploid. This is, I believe, a necessary criterion (not complied with in the previously mentioned cases) before it can be admitted that a mutant gene and its normal allelomorph are doing the same thing to different degrees. Shaven adds up to wild type, but in the triploid, where more is necessary to produce the wild type effect, shaven subtracts from it. This is of particular interest in connection with dominance, and I shall return to it later.

I have carried out the beginnings of a similar series

TABLE 2

THE MANIFESTATION OF THE GENE IN DIFFERENT DOSAGES IN THE DIPLOID AND TRIPLOID

	Haplo IV sv	Diplo II sv	Diplo IV sv/+	Triplo IV sv/sv/sv	Tetra IV sv/sv/sv/sv
2N ...	extreme sv	Type sv	+	slight sv	extra bristles
3N ...	dies	extreme sv	slight sv	Type sv	slight sv

with the fourth chromosome recessive cubitus interruptus. At the moment I have only the comparison of diplo-IV cubitus interruptus triploid and diploid to offer, but they suffice to show a difference in principle. In this case the triploid is definitely less extreme than the diploid, and it follows that cubitus interruptus is not a lesser normal allelomorph, but behaves differently in some way, a result also arrived at by Dubinin and Sidorov (1934) on other grounds. This is presumably like the case of ebony reported by Muller, League and Offerman, a gene competing with the wild type. Certain data on additive effects at the yellow locus lead to similar conclusions—of particular interest in connection with the work of Graubard (1932) on tyrosinase.

The case of shaven gives a certain insight into the problem of dominance, which may be extended by mention of similar examples. In the case of shaven, quite in accord with the view of Mohr (1933), the dominant effect in the triploid with two fourth chromosomes is due to an insufficiency of shaven material, so to speak. What of other dominants? Do they behave like shaven and simply add up to the wild type? Muller, League and Offerman (1932) have been concerned, in a number of cases, with the suppressions caused by the addition of duplicating fragments. I have been interested in the comparison of the behavior of such dominants in the triploid and in the diploid with extra, duplicating, fragments. I have observed the manifestation of about thirty, in single dose in the triploid. It appears that in all cases the manifestation in triploid is less than in the

diploid; but there are two levels, one of which is almost complete suppression, the other a slight suppression. Those dominants completely suppressed include the known deficiencies; but even here the longer the deficiency, the more likely the appearance of an effect. Now in this result, two things are obviously mixed up, which can be unraveled by the comparison with the duplication-bearing diploid. One is the dosage relation at the locus studied, the other is the question of threshold outside the locus. The comparisons which have been made show clearly that, at the level of complete suppression, the effect is due to the locus concerned. But when the slight suppression is considered, it is the rest of the system which exerts its effect. This is particularly clear in the case of the fourth chromosome dominants, in which triploids and diploids can be compared which have the same relations as concerns their fourth chromosomes.

It is evident from these experiments that in the cooperation of genes, as was already clear from combination experiments, all loci are concerned, but each effect is specific. There is emerging a method for the study of this specificity in *Drosophila* which I have time to touch on but briefly here. I refer to the position effect hypothesis, according to which the developmental effects of genes are conditioned by their neighbors in the chromosome. First advanced by Sturtevant (1925) to account for results at the Bar locus, it has received new impetus from the results of x-ray experiments, in which rearrangements of chromosome are found to be accompanied by mutant like changes at the locus of rearrangement. It is clear that where demonstrated this presents a type of interaction which leads us directly to the primary processes in which we are interested.

In the foregoing I have selected from the *Drosophila* experiments certain ones with which I am most familiar, which seem to me to contribute to the understanding of how genes are concerned with development in this animal. It may be well to reconsider now what we mean by under-

standing how genes influence development. It is the very initiation of those differential processes which determine the formation of the embryo in the egg, and the organ within the embryo and the details of the organ within itself, in which we are interested (*cf.* Morgan, 1934). But most of our genetic characters are characters of detail, and most of the embryological studies are made on the early processes. These are matters of experimental convenience, but they are probably largely responsible for the difficulties that are obvious in establishing a relation between the two fields.

A plausible description of gene action has been offered by Goldschmidt (1927) and taken over by others. In outline it is as follows: Genes produce enzymes, which catalyze reactions. These go on, in the developing organism, at specific rates relative to each other. Any change in the gene produces a difference in the effective enzyme quantity, and therefore will produce definite changes in reaction rate—usually, although not necessarily, in a linear relation to the effective enzyme quantity. These changes in rates of specific processes relative to the rates of others produce as end effects the characters with which we deal in genetics. And such relations are as well responsible in the normal course of events, for the exquisite adjustments studied by the embryologist. On these relatively simple assumptions, it is clear that all the phenomena which I have been describing—manifold effects, eye color development, interactions of all sorts, dominance, and particularly the quantitative relations of genic balance, may be described. But it is necessary then to ask what simplification has been introduced by the description and what does the description have to do with gene action?

It is clear that the discussion in terms of rates is a necessary restatement of the facts. Any description of an embryological process must be, and always has been, a description in terms of rates albeit in disguise. But it then follows that the analysis of gene effects in terms of rates is simply a description of their embryology, and is

quite necessary but by no means sufficient. In point of fact, the inference that since genes affect the rates of developmental processes, they must therefore be catalysts of some sort, is one of those apparently obvious deductions which serve to hide the real nature of the problem. In the first place, there are many kinds of catalysts, acting in widely different ways. Secondly, it is not difficult to show that almost any type of change in a developing organism must result in a rate change if development continues abnormally, simply because development occurs in time. If this reasoning is correct, it follows that the two portions of Goldschmidt's physiological theory of heredity have no connection with each other; that the experimental evidence is all for the descriptive embryological portion; and that the other does not serve as a basis for experiment, since it is not closely enough defined.

This is not to underestimate the value of Goldschmidt's realization that if the study of how genes influence development is to be pursued, we must study development. But we know that now, and it is rather necessary to test the other side of the story. A theory of the influence of genes on development must have some concrete qualities. It must be concerned with how and where and when the chromosomes produce their effects upon the cytoplasm. One may even guess that until we know something about how genes reproduce, we are not likely to know very much in principle about how they affect development.

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PAPERS READ AT THE PITTSBURGH MEETING OF THE GENETICS SOCIETY OF AMERICA

PROFESSOR P. W. WHITING
SECRETARY

1. *Further Evidence of Physiological Mutations in the Cladoceran, Moina macrocopa:* ALBERT A. BARDEN, JR., and THELMA R. WOOD, Brown University. By inbreeding within Banta's Line 1012, previously parthenogenetic for 1,250 generations, 100 animals were hatched from the sexual eggs obtained. These sexually produced individuals were compared as to reproductive capacity and longevity with 100 parthenogenetically produced animals from the same line, each set out on the same day and kept under the same conditions as the corresponding sexually produced animal. Of the 100 sexually produced individuals 58 died early, as against 6 of the parthenogenetically produced animals; 27 of the sexually produced animals and 91 of the parthenogenetically produced animals produced young. Statistically significant differences between the sexually produced and parthenogenetically produced animals favoring the latter were obtained as to age at first brood, average number of young per brood, average number of young per mother, average reproductive index and average length of life. A comparison of the results of the present experiment with those relating to Line 1012 in Wood and Smith's experiment of 1932 shows that the sexually produced individuals obtained in the present experiment lived only about half as long (5.1 days as against 10.33 days) and fewer were fertile (27 per cent. as against 72.2 per cent.). The fertile animals averaged only 80.85 young compared to 120.28 young for the earlier experiment. It seems likely that during the 160 parthenogenetic generations elapsing between the former and the present experiments lethal or semi-lethal mutations occurred which, made homozygous by inbreeding, exerted an injurious effect on the viability, reproductive capacity and longevity of the sexually produced offspring of this clone.

2. *The Racial Factor in the Response to a Hormone, Prolactin:* ROBERT W. BATES, OSCAR RIDDLE and ERNEST L. LAHR, Carnegie Institution, Station for Experimental Evolution. It has been established that different races (and hybrid types) of

doves and of common pigeons give unequal responses (crop-gland weights) to the same quantity of the hormone prolactin. The study is based largely on "endocrine" races established during the past 14 years in our bird colony. Groups of pigeons of different races have been found to differ as follows: With full correction for differences in body weight White Kings give a response four times that of Tippler pigeons; they differ by an equivalent amount from hybrids between the Tippler and one of our derived races. Among ring doves race 63 gives a unit response with one sixth the amount of prolactin required for the same response in race W. Again, one type of hybrid dove (N2 × 62) gives a unit response with one fifth the amount of prolactin required by hybrid 63 × 72, and one eighth that required by race W. Many other racial and hybrid types were studied. The influence of other factors than race on the intensity of response to prolactin has been studied. Age of animal —particularly the distinction of immaturity and adult life—is of great importance. A difference of as much as tenfold has been observed. Individual variation of response also occurs within long inbred races. A physiological or genetic basis of the variable response has not yet been found. Such racial and individual differences in response to the hormones of the body probably have important consequences in the survival and evolution of vertebrates.

3. *Arrangement of Chromosome Ends in Datura discolor:* A. D. BERGNER and A. F. BLAKESLEE, Carnegie Institution of Washington, Cold Spring Harbor, N. Y. Interspecific crosses have been made using the pollen of *Datura discolor* on the tester races of *D. stramonium, D. ferox* and *D. quercifolia. D. stramonium* × *D. discolor* gave a hybrid which showed a circle of ten chromosomes plus seven bivalents at metaphase I in PMC. *D. ferox* × *D. discolor* showed a circle of ten chromosomes (or a circle of six to which a circle of four was attached) plus seven bivalents, including one pair of unequal chromosomes. *D. quercifolia* × *D. discolor* showed a circle of six chromosomes plus a circle of four plus seven bivalents, including one pair of unequal chromosomes. By means of these crosses and crosses to prime type tester races of *D. stramonium,* the five chromosomes which differ from those of Line 1 of *D. stramonium* were determined as being 1 · 11, 2 · 17, 12 · 22, 15 · 21 and 16 · 18 instead of 1 · 2,

11 · 12, 15 · 16, 17 · 18 and 21 · 22. Segmental interchange between non-homologous chromosomes apparently has played a part in the origin of this species as well as in the origin of those previously studied.

4. *Crossability in Relation to Taxonomic Classification in the Genus Datura:* A. F. BLAKESLEE, M. J. MURRAY and S. SATINA, Carnegie Institution of Washington, Cold Spring Harbor, N. Y. Aside from the tree *Datura* (Brugmansia) and the monotypic group formed by the aquatic species *D. ceratocaula,* taxonomists recognize two sections in the genus *Datura.* The first includes those with erect, four-valved, persistent capsules (*D. stramonium, D. ferox* and *D. quercifolia*). These are fertile *inter se.* The second section includes species with more or less nodding capsules (*D. leichhardtii, D. pruinosa, D. discolor, D. innoxia, D. meteloides* and *D. metel*). *D. leichhardtii* acts as a bridging species, since it enters into crosses with species in both groups, but only when it is used as a female. *D. discolor* enters into crosses with members of the *stramonium* group, but only when used as a pollen parent. Thus, crossability fails to run parallel with taxonomic relationships as established by sections in the genus, and crossability may be different in reciprocal matings. Incompatibility between species may be shown by failure to set capsules or by arrest of seed development at various stages. In the cross *D. stramonium* × *D. metel,* normal fertilization was observed with 2n zygotes and 3n endosperm. Embryos were formed with up to eight cells, and endosperm development continued in some cases for about two weeks. Later development was not observed.

5. *Sex-linkage as Shown by Biparental Males in Habrobracon:* C. H. BOSTIAN, North Carolina State College of Agriculture and Engineering. Sex-linkage of the gene fused as shown by counts of females (heterosyngamic combinations, XY) has been previously reported.[1] Experiments demonstrating sex-linkage of the same gene as shown by counts of biparental males (homeosyngamic combinations, XX and YY) are summarized herewith. Orange-eyed females heterozygous for fused, o/o X/fu.Y, were crossed with closely related black-eyed fused males, both fu.X and fu.Y. The crosses with fu.X males yielded females, 96 wild-type, +/o Y/fu.X, and 506 fused, +/o fu.Y/fu.X (15.9 per cent.

[1] P. W. Whiting, THE AMERICAN NATURALIST, 68: 68.

crossovers), and biparental males, 92 wild-type, +/o X/fu.X, and 16 fused, +/o fu.X/fu.X (14.8 per cent. crossovers) as well as azygous males, 220 orange and 197 orange fused. The crosses with fu.Y males yielded females, 1,575 wild-type, +/o X/fu.Y and 193 fused, +/o fu.X/fu.Y (10.9 per cent. crossovers), and biparental males, 41 wild-type, +/o Y/fu.Y and 230 fused, +/o fu.Y/fu.Y (15.1 per cent. crossovers), as well as azygous males, 577 orange and 510 orange fused. The deficient (crossover) class among the biparental males thus proves to be of the type opposite to the deficient (crossover) class among the females, as was to be expected.

6. *Studies of Normal Salivary Chromosomes of Drosophila melanogaster:* CALVIN B. BRIDGES, Carnegie Institution of Washington (address: California Institute of Technology, Pasadena, Calif.). Following the work of Heitz and especially of Painter the use of the 100-fold longer chromosomes of the salivary glands is essential in the analysis of chromosome aberrations. Two prerequisites are, first, accurate detailed maps of all the normal salivary chromosomes against which to check the precise point of break or area of disturbance; and second, an objective method of referring to particular "bands" or sections of those chromosomes.

For intensive study several refinements of technique are required, including (1) relatively light transparent staining of the especially thick salivary chromosomes found in the race "giant," (2) picking only stretched chromosomes for examination, (3) using 120 obj., 10 oc. with the light carefully controlled as to numerical aperture and focus of achromatic condenser, area illuminated, intensity and color-contrast, and (4) checking with oblique light directed along the axis of the chromosome. The resulting maps will be shown as large to-scale drawings (exhibits section) and as lantern slides. The identification bandmarks and special features of X, 2L, 2R, 3L, 3R and 4 will be emphasized. Nearly 600 "lines" have been observed for the X and over 3,000 for the full set, counting "doublets" and "capsules" as two lines.

The proposed system of reference is to divide the maps into sections numbered 1–20 for X, 21–40 for 2L, 41–60 for 2R, 61–80 for 3L, 81–100 for 3R and 100–101 for 4. Each section begins at one of the most distinctive heavy bands, and each subsection with a clear band.

7. *The Structure of Salivary Chromosomes and the Relation of the Banding to the Genes:* CALVIN B. BRIDGES, Carnegie Institution of Washington (address: California Institute of Technology, Pasadena). Painter showed that sections of consecutive mapped loci correspond to sections of "bands" of the salivary chromosomes. In testing the relation between single loci and salivary structures I found that two lethals (*1*–2ax and *1*–N) lie in a space showing 2 "bands," while the next locus (speck) lies in the adjacent space showing 2 bands, one presumably corresponding to an unmapped locus. The conclusion that there is a one-to-one relation between loci and bands has further support from a detailed census of the bands. Over 3,000 distinct "lines" were observed, of which fully 80 per cent. exist in pairs. If one includes under "bands" the "capsules," the pairs of equal intensity lines, the "singlets," and the lines of dots, dashes or vesicles, this number corresponds to about 1,800 "bands," in good agreement with calculated totals of loci. In a paper sent to press in June I showed that salivary chromosomes are cables of 8 + 8 strands, derived by successive divisions of the 2 homologues, a conclusion reached also by Koltzoff (*Science,* October 5, 1934). Study of the different types of "bands" leads to the conclusion that in the centers of the 16 dots or vesicles of the "singlets" lie the 16 genes of that locus. The genes at many loci are the centers of heavier deposits of chromatin, which is bipolar in arrangement with coalescence at the edges, thus leading to a pair of lines enclosing the achromatic genes. With still heavier chromatin the paired lines become thick plates joined at their edges in "capsule" form around the genes.

8. *Conditions of Pollen-Tube Growth Found in Pollinations Between Ten Species of Datura:* J. T. BUCHHOLZ, L. F. WILLIAMS and A. F. BLAKESLEE, University of Illinois. Tests have been made of pollen-tube growth in the ninety possible crosses between ten herbaceous species of Datura. These were compared with the tests of the ten species in sib-crosses and self-pollinations. Repeated tests over a period of years at various times of season, including greenhouse plants as well as field-grown plants, have enabled us to select the tests which showed the best possible conditions obtained in each type of pollination. The selected tests were studied and compared statistically for percentage of pollen germination, distance and rate of growth, and for abnormalities in pollen tube growth, such as the propor-

tion of burst pollen tubes in the style. It is possible to rank the styles of the ten species with reference to their ability to grow the pollen of other species and also to rank the pollen of each species with reference to its ability to germinate and grow in the style of each of the other species. Nearly all combinations are more or less favorable if the cross is made in one way and give very abnormal conditions in the reciprocal cross. It is therefore possible to determine which combinations of the crosses are obviously impossible, but many crosses giving good pollen-tube growth, which should make fertilization possible and actually develop fruits, still result in failure to obtain viable seeds.

9. *Abnormal Genetic Ratios in Chromosome 1 of Maize Associated with Pollen Abortion:* CHAS. R. BURNHAM, University of West Virginia. In an inbred line, plants showing an average of about 50 per cent. of pollen abortion were found which showed no ovule abortion. The pollen sterility is transmitted only when such plants are used as the female parents in crosses. Tests using the pollen of plants heterozygous for this sterility and for factors in different linkage groups have given abnormal ratios only for factors in chromosome 1. Normal allelomorphs of these factors are deficient in numbers. This is to be expected if the "factor" producing the aborted pollen is located in chromosome 1 at a locus near these genes. The results for the P p (colored pericarp *vs.* colorless) factor pair fit a recombination per cent. of 20.7 between *P* and *sterility*. The results for the F f (normal *vs.* fine striped) pair fit a recombination per cent. of 34.5. The direct backcross test, using the partially sterile plant as the female parent, shows that the sterility "factor" is between *P* and *br* (brachytic), showing 34.5 per cent. recombination with P, 31.9 per cent. with *br*, and independence with bm_2 at the other end of the linkage group. Crosses with two interchanges involving chromosome 1 (1–6a and 1–2a) were made to facilitate a study of chromosome 1 at the prophase stage. As yet, cytological examination has shown no abnormality in any of the chromosomes. A very short deficiency may be present and not easily detectable.

10. *Mutation Rate for Seeds of Datura Buried 22 Years:* J. L. CARTLEDGE and A. F. BLAKESLEE, Carnegie Institution of Washington, Cold Spring Harbor, N. Y. In 446 plants, grown from soil in which seeds of the jimson weed (*Datura stramonium*) had been undisturbed for 22 years, the rate of pollen

abortion mutations was 1.2 per cent. The plants obtained differed from the standard line in color, and possibly also in chromosomal type. The rate of mutation found is low in comparison with that of plants from seeds aged 10 years in the laboratory, and is only slightly higher than the rate from controls grown from fresh seeds. This indicates that age alone is not the cause of the greatly increased mutation rate previously found in aged seeds.

11. *Behavior of Extra Chromosomes in an Oenothera from California:* RALPH E. CLELAND, Goucher College. Seed from Mono County, California, yielded two plants with extra chromosomal material. This is the first time that extra chromosomes have been found in wild Onagra. One plant had, in addition to seven pairs of normal chromosomes, three half-sized chromosomes, attached end to end, or forming a ring-and-rod. Each had an attachment point, and disjunction followed the usual procedure for trivalents. The other plant had two extra half-sized chromosomes, which paired normally, and disjoined with great regularity. Each microspore contained as a rule seven normal chromosomes and one half-sized chromosome. As might be expected, later generations derived by selfing from the latter plant have continued to show the extra pair. The situation in outcrosses, however, has proved anomalous. Although the expected chromosome number in all such cases is fourteen normals plus a single extra, most of the hybrids between the original plant with eight pairs and races with normal chromosome number have shown a *pair* of extras, like the "Mono" parent. This situation must have been brought about by reduplication of the extra, either in a gametophytic mitosis or in the zygote. All hybrids with two extras showed normal pairing and disjunction of the extras. The extras showed no affinity for other chromosomes, as shown by their failure to take part in the formation of multivalents. They were both shorter and narrower than normal chromosomes, had approximately median spindle attachment and showed terminal association at both ends in forming pairs.

12. *Rôle of Genes in a Cell:* M. DEMEREC, Carnegie Institution of Washington, Cold Spring Harbor, N. Y. Thirty-five deficiencies, involving thirteen regions of the X-chromosome, were tested for cell-lethal effect by the autosomal minute method by which a high frequency of somatic segregation of X-chromosomes

is produced. If both X-chromosomes are marked, then each of the twin spots produced by such segregation shows characteristics of one marked chromosome. If a cell-lethal deficiency is introduced in one of the chromosomes only one spot appears where two are expected. All deficiencies tested involve a minute region of the X-chromosome, as evident from both genetic and cytological (salivary chromosome) tests. Many of them include one known locus only and some one salivary chromosome band only. Of the thirteen regions tested cut is the only region found not to be cell-lethal. This suggests that at least the majority of genes function in every cell of the organism and moreover that the presence of the majority of them is essential in vital functions of every cell.

13. *A Unique Chromosome Group in a Species of Drosophila:* TH. DOBZHANSKY, California Institute of Technology. A species of Drosophila, as yet undescribed, closely related to *Drosophila pseudoobscura* Frolova, occurs on the Olympic peninsula, Vancouver Island, and the adjacent portion of the mainland. In this species, the male has an uneven number of chromosomes ($2n = 9$): two pairs of rods, one pair of dots, one rod having no mate, one larger and one smaller V-shaped chromosome. The female has three pairs of rods, one pair of dots, and one pair of V-shaped chromosomes ($2n = 10$). The larger V and the unpaired rod in the male are the X-chromosomes (X′ and X″), the smaller V is the Y-chromosome. Thus, male is X′X″Y, and female is X′X′X″X″. At meiosis in the male the X′ pairs with the Y, while X″ remains unpaired, and at the first division passes undivided to one of the poles. At the second division X″ splits equationally. The new species, when crossed to *Drosophila pseudoobscura,* produces visibly abnormal sterile males and females more normal in appearance that deposit eggs, most of which fail to hatch. A number of interesting possibilities for studying the problems of sex-determination and the nature of interspecific differences seem to be opened.

14. *Gametophytic Genes in a High Waxy Strain of Maize:* WILLIAM H. EYSTER, Bucknell University. In certain pedigreed cultures, maize plants heterozygous for waxy endosperm have been found to give rise to F_2 kernel progenies in which the per cent. of waxy kernels varies from 5 to 48, and to backcross progenies having from 39 to 90 per cent. of waxy kernels. Reciprocal

crosses between such heterozygous plants and homozygous waxy plants indicate that in all cases the deficiency of starchy and excess of waxy kernels or the reverse are caused by genes affecting the microspores of male gametophytes but never by megaspores or female gametophytes. Studies of the pollen and of the kernel progenies produced when the pollen of individual plants is applied to the silks of homozygous waxy plants revealed an extensive polymorphism in the pollen. Some of the genes which render the male gametophyte non-functional have their loci in Chromosome 9 and are responsible for the observed deviations from the expected numbers of waxy and non-waxy kernels.

15. *A Study of Segregation in Mutual Translocations in Drosophila melanogaster:* H. BENTLEY GLASS, National Research Fellow, University of Missouri. The frequency of orthoploid and aneuploid gametes was determined in three dominant mosaic allelomorphs of brown, in each of which there is a mutual translocation involving chromosomes 2 and 3, 2 being broken at brown, 3 just to the left of the spindle-fiber attachment. The three cases result alike in 55 per cent. of orthoploid gametes and 45 per cent. of the aneuploid type resulting from disjunction along the longer axis of the cross formed when all homologous parts are in apposition. The aneuploid type resulting from disjunction along the shorter axis amounts to less than 1 per cent. The similarity of the three cases led to tests showing that they are broken not only at identical points in 2, but also in 3. Since they are of independent origin, this is regarded as a certain indication of weak spots in the chromosome especially liable to breakage. Segregation was tested in individuals carrying two mutual translocations so related that the appositional figure was an acentric cross with equal axes. The two types of aneuploid gametes are again equal, but a considerable increase in the proportion of orthoploid gametes (63 per cent.) is observed for the first time, all previous effects of inversions and asymmetrical configurations having resulted predominantly in shifting the proportions of the two aneuploid types. The slight increase of orthoploidy found when inversions are present along one axis is not exaggerated but decreased by placing inversions along the other axis also. Three new principles of segregation in compound chromosomes may now be formulated: (1) Acentricity of the cross produces a reduction of the aneuploid types and an increase of directed segregation. (2) Inequality of the axes of

the cross produces a proportional inequality of the aneuploid types. (3) Inversions along one axis of the cross produce an inequality of the aneuploid types; but along both axes, their opposing effects neutralize each other.

16. *Genetics of Xiphophorus, the Swordtail Killifish; I, the Inheritance of Micromelanophores:* MYRON GORDON, Zoological Laboratory, Cornell University. The basic color of the wild-type Mexican swordtail (*Xiphophorus hellerii*) is gray to olive green. This color is due to integumentary xanthophores and melanophores. Both chromatophores are small and exceedingly numerous. The gold swordtail appeared recently in the cultures of commercial fish fanciers. Its genealogy is obscure. It may have been developed by crossing the common swordtail to the golden mutant of *Platypoecilus maculatus,* back-crossing to the swordtail and then inbreeding the back-cross hybrids, selecting only yellow swordtail-like fishes. When the golden swordtail (no micromelanophore *stst*) is crossed to the wild type (stippled with micromelanophores, *StSt*) the stippled type appears ($F_1 = 230$ *Ststt*); when stippled F_1 are back-crossed to recessives, the ratio is 227 *Ststt* to 140 *stst;* when F_1 are inbred, the ratio is 68 *St* to 22 *st.* The distribution of the sex indicates that *St* is an autosomal factor. The deficiency of golds in the ratios is attributed to the well-known weakness of the golden swordtail.

17. *Genetics of Xiphophorus, the Swordtail Killifish; II, the Inheritance of the "Montezuma" Characters:* MYRON GORDON, Zoological Laboratory, Cornell University. The aquarium fish known as the "montezuma" swordtail is not *Xiphophorus montezumae* Jordan and Snyder. It is, originally, a hybrid between one of the *spotted* sub-species of *Xiphophorus hellerii* Heckel and *Xiphophorus montezumae.* Many original hybrid characteristics are lost, due to constant back-crossing to *X. hellerii.* The aquarium-developed "montezuma" swordtail is identified by an all-over color of deep orange to red (with the exception of the throat area) and several rows of large black spots. The wild *X. hellerii* is grayish green; the wild *X. montezumae* is grayish brown. The aquarium-developed "montezuma" swordtail was crossed to the yellow swordtail, an aquarium-produced strain of *X. hellerii.* The characters, in opposition, between the "montezuma" and yellow swordtails are: 1, orange to red body color (factor *Rf* for erythrophores); 2, rows of large black spots (fac-

tor *Sp* for macromelanophores); 3, microscopically detected small black pigment cells (factor *St* for micromelanophores). The "montezuma" is *Rf Sp St* while the yellow swordtail is *rf sp st*. The F_1 were of four types: two parental types, No. 1, reddish with rows of black spots, "montezuma" (*Rfrf Spsp Ststst*) and No. 2, yellow (*rfrf spsp ststst*); two new types, No. 3, the wild grayish green (*rfrf spsp Ststst*) and No. 4, reddish with only a trace of black spotting near the head (*Rfrf Spsp ststst*). The inhibiting action of *st* upon the expression of *Sp* is similar to that of analogous factors in *Platypoecilus maculatus*. When type No. 4 was crossed to a homozygous No. 3 type, type No. 1 was synthesized: *Rfrf Spsp ststst* × *rfrf spsp StStSt* = *Rfrf Spsp Ststst* + *rfrf spsp Ststst*. The "montezuma" character, *Mo* of Kosswig, is a complex of three factors: *Rf* and *Sp* are sex-linked probably; *St* is autosomal.

18. *Recessive Genes in Geographic Races of Peromyscus:* R. R. Huestis and Elizabeth Barto, University of Oregon. Continued breeding of the offspring of *P. m. gambeli-rubidus* hybrids has led to the discovery of a congenital tremor, lethal in all five cases to date within the first month of life, and two new characters which modify the color of the pelage. One of these produces a reddish brown, the other a clay-colored pelage; both carry reduced pigment in ears and tail stripe. In addition an albino *P. m. rubidus* has been taken in the field.

19. *Chiasma Frequency in Relation to the Mechanism of Cancer Susceptibility:* C. Leonard Huskins and E. Marie Hearne, Department of Genetics, McGill University. Counts of chiasma frequency have been made on six strains of mice, obtained from Dr. C. C. Little, as an indirect, but relatively precise, numerical test of two related working hypotheses on the mechanism of susceptibility to the development of spontaneous tumors. (Direct tests involve a larger subjective factor.) Three resistant strains have been found to have a significantly higher chiasma frequency than two which are highly susceptible and one strain is intermediate. The differential frequency is distributed throughout the chromosome complement. The apparent significance of the results is discussed.

20. *Chromatid Translocations and Other Effects of X-radiation on Trillium Chromosomes:* C. Leonard Huskins, A. W. S. Hunter and Alma C. Howard, Department of Genetics, McGill

University. Results differing considerably from those of previous cytological studies of the effects of x-rays have been obtained. In several respects they are in closer accord with genetic results.

21. *The Spiral Chromonemata of Trillium erectum:* C. LEONARD HUSKINS and STANLEY G. SMITH, Department of Genetics, McGill University. The apparent double or single nature of chromosome spirals and the establishment of changes of direction are affected by the time relationship between "terminalization" and the beginning of coiling. Changes in direction of coiling bear a facultative relationship to chiasmata. A heterogonic growth hypothesis for the mechanism of coiling is presented, and examined in relation to the changes of direction. There is no evidence of a minor spiral within the major chromatid spirals of *Trillium.* Observations on "bridge" chromosomes indicate that some of the evidence adduced in favor of the minor or "primary" spiral concept in other plants may be due to misinterpretations.

22. *On the Physiological Basis of Genetic Resistance to Salmonella pullorum in the Fowl:* F. B. HUTT, University of Minnesota (now at Cornell University). The percentage of reactors to the agglutination test for *S. pullorum* is significantly lower in White Leghorns than in various heavier breeds and breeders find Leghorns comparatively resistant to the disease it causes. In an attempt to find the physiological bases for this resistance, males heterozygous for the dominant white of White Leghorns were matched to black fowls and their progeny treated with uniform doses of *S. pullorum.* Mortality rates to three weeks of age were practically equal in the *I* and *i* chicks, indicating the absence of genetic linkage or physiological association of resistance with the *I* gene. Body temperatures of White Leghorn chicks did not differ from those of Rhode Island Reds up to three days after hatching, but on the sixth and ninth days the mean temperatures of White Leghorns were higher by 0.871° and 0.944° F., respectively. The differences were consistent in six series, including 357 chicks and are statistically significant. Blood counts at six to ten days showed the erythrocyte and leucocyte counts to be significantly higher in Rhode Island Reds than in White Leghorns. In the latter breed lymphocytes were higher and polymorphonuclear neutrophiles lower than in Rhode

Island Reds, but the two breeds did not differ in basophiles and eosinophiles.

23. *Complementary Action in a Generic Hybrid of Genes having a Biochemical Effect:* M. R. IRWIN, University of Wisconsin, Madison, Wisconsin. Hybrids between Pearlnecks (*Spilopelia chinensis*) and Ring doves (*Streptopelia risoria*) possess in their erythrocytes biochemical substances not found in the cells of either parent. Present evidence indicates that the "hybrid" substances are produced by the interaction of specific genes from each of the parental species. Examination of the cells of ten other species of Columbidae has shown the presence of the hybrid substances, at least in part, in three, *viz., Streptopelia damarensis, Stigmatopelia senegalensis* and *Columba rufina.* Presumably in these species there occurs normally the same combination of genes which in the Pearlneck-Ring dove cross produces the "hybrid" substances.

24. *A Genetic Interpretation of Categories Higher Than the Species:* ALFRED C. KINSEY, Department of Zoology, Indiana University. An attempt is made to correlate a taxonomic study of the gall wasp genus *Cynips* with the data of genetics. In previous studies the author has shown species to be realities which are large populations representing interbreeding Mendelian races having access to a common stock of genes. The field data confirm the geneticist's interpretation of the origin of species by mutation or hybridization, while emphasizing the necessity of the isolation of the new from the parental stocks. In the present studies, higher categories (complexes, subgenera, genera) appear to be artificial conventions convenient for cataloging the diverging ends of a phylogenetic series. Degree of difference, time or mode of origin and stability of diagnostic characters do not justify their recognition. The higher categories of *Cynips,* established for the ends of the evolutionary lines within the United States, are united by a long list of newly discovered Mexican species into one continuous albeit much divided chain. The evolutionary pattern is not that of a tree of life, with the older, main branches disappearing in the newer tips. The pattern is that of a dichotomously dividing vine in which there are no main stems, and in which the older may remain coexistent with the younger leaves (species). If the end products are called genera, it must be recognized that the divi-

sions between the groups are arbitrary, inasmuch as the first species in one "genus" may be no further removed from the parental species in the other, than any two closely related species within the same "genus."

25. *Circling, a Two-Gene Trait of the Mouse:* T. LAANES and E. C. MACDOWELL, Department of Genetics, Carnegie Institution of Washington, Cold Spring Harbor, N. Y. The trait circling combines the horizontal whirling of Japanese waltzers and shakers with normal head movement and violent response to sounds, such as erratic leaps and somersaults which diminish with age, while the whirling continues throughout life. In the inbred strain 17 per cent. normal overlaps occur. First generation by a normal strain gives all normals. Back cross, observed 244 normals: 65 circlers; expected (correcting for normal overlap) 64.9 circlers. F_2 observed, 184 normals: 8 circlers; expected (correcting for normal overlap) 10 circlers. Thus circling appears to depend upon the combined action of two recessive genes.

26. *Experiments on Hybrid Vigor in Maize:* E. W. LINDSTROM, Iowa State College. A biometric analysis of F_1 and F_2 generations from crosses of inbred lines of maize, dealing with ear weights (yield) and some of the components of ear weight, such as ear length, diameter and number of kernel rows. The F_2 curves of individual ear weights are not markedly skewed, but the constituent parts of ear weight do exhibit great skewness. Both ear length and diameter give positive skewness, indicative of dominance of large size, whereas number-of-row curves show negative skewness. The balance between these positive and negative trends is reflected in the decreased skewness of the ear weights themselves. Multiple correlation studies afford verification for these relationships. Hence the absence of skewness is not an indication of lack of dominance in size (weight) genes. Ashby's simple hypothesis of heterosis is subjected to a critical test in which the "capital" of the young hybrid plants is greatly reduced by decapitation. Despite this handicap the hybrids overtake the growth of the untouched parental lines, proving beyond reasonable doubt that the hybrid has a much faster rate of growth than the parental strains.

27. *Maternal Influence in the Inheritance of Spontaneous Mouse Leukemia:* E. C. MACDOWELL, Department of Genetics,

Carnegie Institution of Washington, Cold Spring Harbor, N. Y. Reciprocal crosses have been made between a "leukemic" strain of mice (incidence 90 per cent.) and a "negative" strain (incidence 1 per cent.) followed by back crosses to the "negative" strain. When transmitted through the males the incidence of leukemia is closely correlated with the total heredity from the leukemic strain; when transmitted through the females the incidence is significantly higher. In F_1 the increase is 18.9 per cent. ($=4.4 \times$ P.E. $140+106$ mice); in BC the increase is 26.6 per cent. ($=7.0 \times$ P.E. $97+156$ mice).

28. *The Localization of Nine Gene Loci on the X-Chromosome of Drosophila melanogaster:* OTTO MACKENSEN, University of Texas. Deletions of known genetic make-up have been studied by the salivary gland method, and these together with available translocations have made it possible to place the following gene loci within very narrow morphological limits; prune, white, facet, split, echinus, cut, vermilion, forked and Beadex. The locus of white is restricted to the area on or about one narrow line, and vermilion lies in the left-hand part of a complex band. The other genes are restricted to areas covered by several bands. So far, the evidence suggests that the genes lie within the chromatic areas.

29. *An Analysis of the Temperature-Effective Period for Facet Determination in Bar-eyed Drosophila:* OTTO S. MARGOLIS, Washington Sq. College, New York, N. Y. Data on the temperature-effective period (T.E.P.) for facet determination at 28° are presented for Bar and Bar vestigial stocks. It is demonstrated through suitable experiments that individual variability in the temporal position of the T.E.P. exists. As a consequence, the T.E.P. as measured experimentally does not represent the mean duration of the period per individual, but rather the time limits from the beginning of the T.E.P. in the first individuals of the population to the termination of the period in the last. It can be demonstrated that no rigorous analytical resolution of the T.E.P. curves in their component elements is possible without independent information concerning either the time-course of facet determination or the curve expressing the variation in time at which the T.E.P. of the individuals in the population occurs. Through the use of suitable assumptions a minimum value of the mean individual T.E.P. is obtained. An explanation for the

generally observed sigmoid character of the T.E.P. curves is proposed.

30. *Recessive X-ray Lethals in Habrobracon:* JANE MAXWELL, Carnegie Institute of Technology. Virgin females from wild-type females crossed with cantaloup gynoid males produced males segregating in 1:1:1:1 ratio. Among 16,217 F_2 males from P_1 females x-rayed (2,500 r) and among 6,328 from controls there was no deviation in this ratio associated with treatment, nor was there any reduction in fecundity of the F_1 females. In an experiment in which some of the P_1 males were treated with a series of dosages, 27 F_1 females from untreated males produced 4,713 males with an average per day per female, 7.06; 23 from males treated with 2,500 r produced 3,516 with average 4.59; 27 from 3,000 r group produced 2,194 with average 3.11; 23 from 3,500 r produced 1,832 with average 2.53; 20 from 4,000 r produced 1,528 with average 2.57; and 12 from 4,500 r produced 971 with average 2.86. Twenty-one females from controls each averaged six or more offspring per day, while only two averaged three or less. Thirty-one females from treated averaged six or more, while 64 averaged three or less. The arrays were markedly bimodal for each of the treated groups. Recessive lethals were therefore produced by treatment of sperm. The 27 fraternities from controls had percentages of cantaloup and of gynoid not significantly different from 50 per cent., while 101 from treated showed no significant deviation for cantaloup and 101 showed none for gynoid. Four from treated with less than 17 per cent. cantaloup and four from treated with less than 38 per cent. gynoid deviated significantly ($P = .99+$) below 50 per cent. in their percentages. Some of the lethals therefore showed linkage with cantaloup and some with gynoid. A single mutant male (lemon) occurred in an F_2 fraternity of the 3,500 r group. One mosaic and four "freaks" occurred among total control, 11,041, of both experiments, while six mosaics and nine "freaks" occurred among total treated, 26,258.

31. *Pigmental Asymmetry and Chromosome Deletions Resulting from X-radiation in Habrobracon:* R. A. MILLER, State University of Iowa. Offspring from females homozygous for various recessive traits crossed with males recessive for others were x-rayed (500 r to 4,000 r) as eggs or as larvae. Among 2,790 females, 23 diploid males and 1,664 haploid males there occurred

five mosaics. Two of these were gynandromorphs with female parts wild-type and male parts showing recessive maternal traits. One female (from orange female by white stumpy male) (3,500 r) had white region dorsally in left eye (15 facets), left ocellus white, median and right ocelli mixed. One of two females (from white cantaloup female by shot-vein aciform male) (2,500 r) had right gonapophysis aciform while the other had left gonapophysis aciform and a recessive (white or cantaloup?) sector ventrally in the right eye. Pigmental asymmetry in integument occurred among 418 females, 2 diploid males and 102 haploid males. Observations were made at 16 different regions of muscle fiber attachment and showed asymmetry as follows: ocellar, 42 asymmetrical; occipital, 128; median mesonotal, 65; lateral mesonotal, 318; prosternal, 10; mesonotal, 28; first abdominal tergite, 9; second, 36; third, 18; fourth, 10; fifth, 0; first abdominal sternite, 5; second, 14; third, 14; fourth, 5; fifth, 1; total, 703. For each region left or right side was darker with equal frequency. 14.2 per cent. treated (3,500 r) as larvae but only 3.7 per cent. treated (3,500 r) as eggs were asymmetrical. Females showed greater asymmetry than males at the higher dosages and asymmetry increased with strength of dosage given the larvae (4,000 r, – ♀♀ 33.6 per cent., ♂♂ 14.1 per cent.; 3,500 r, – ♀♀ 16.4 per cent., ♂♂ 8.6 per cent.; 1,500 r – ♀♀ 5.3 per cent., ♂♂ 7.4 per cent.).

32. *The Inheritance of Albinism in Freesia:* W. P. MORGAN, Indiana Central College. Appearance of albino seedlings in the progeny of hybrid Freesias was first noted, but not studied, by Jacob in England (1923). This character is of the albescent type, in which the leaves of seedlings usually produce a noticeable tinge of chlorophyll near their bases along the mid-rib which, in some, suggests the "pie-bald" character in maize. These plants usually die in the early seedling stage. The testing of more than 50 commercial (clonal?) varieties produced by breeders of America, Holland and England showed 19 varieties to carry this character as a recessive. These varieties had the following general color classification: 12, pink; 3, orange; 1, yellow and pink; 2, white; 1, yellow. Study was complicated by the fact that hybrid Freesias produce few if any seeds when selfed, requiring more generations by less direct methods. Several thousand seedlings from 165 crosses were used in this study

during the years 1929 to 1934, inclusive. Results of this analysis indicated the chlorophyll deficiency of the seedlings to be due to a single pair of genes recessive to those producing normal green plants. The species *F. armstrongii, F. fergusoniae, F. andersoniae, F. hurlingii* and two unidentified species from the locality of Humansdorp, C. P., South Africa, were used in testing heterozygous hybrids. All species were found to be homozygous for normal green. One of these albino type seedlings from crosses of 1931 was grown to maturity. Its older leaves became virescent except at the tips and edges, while the normal color developed at their bases and along the mid-ribs. The few seeds from this weakly, self-sterile plant produced in crosses with heterozygous plants verified the above analysis.

33. *Apparent Gene Mutations Due to the Position-Effect of Minute Gene Rearrangements:* H. J. MULLER (professor of zoology, University of Texas, on leave of absence for 1934–35), A. PROKOFYEVA and D. RAFFEL, Institute of Genetics, Academy of Sciences, Moscow, U. S. S. R. Having obtained definitive evidence, from studies on scute allelomorphs, for the "position-effect" alternative (Muller, 1930) as the explanation of "mutations" accompanying obvious rearrangements, we have examined cases of "double mutation," apparently unaccompanied by rearrangement, to determine whether they might be due to rearrangements having their two breaks too close together for genetic recognition. Cytological examination of salivary gland chromosomes has verified our conjecture, revealing minute rearrangements having their two points of breakage and reattachment in the positions expected. Thus, lethel J1-scuteJ1 is an inversion including only chromomere 1 and part of 2, as we had anticipated since finding scute to be in 2 and lJ1 left of it. Again, achaete3-ommatidial disarrangement is an inversion including the right part of chromomere 2 and chromomeres 3 and 4, in accordance with our knowledge of achaete being in 2 and *om* slightly right of 2. Scute19 is a still minuter rearrangement, without "double mutation" effect. It involves a section only four to eight genes long (Muller, in press), all located in a part of one chromomere, and not inverted but deleted and inserted elsewhere. If this section had been only inverted, the fact would have been indeterminable by linkage or cytology. These and other cases raise the question: Are most or perhaps practically all x-ray "gene mutations" intergenic position-effects rather

than intragenic transformations? And the more momentous question follows: To what extent may natural mutations also be of this type—a type incapable of providing the material for indefinitely continued evolution?

34. *Observations on the Direction of Coiling of Chromonemata as Related to Reduction and Crossing Over:* B. R. NEBEL, N. Y. State Agricultural Experiment Station. (A) Direction of coiling is not a genetical factor. Direction of coiling was studied in meiotic chromosomes of *Medeola virginiana* L., *Tradescantia reflexa* Raf. normal, and *T. reflexa* x-rayed showing rings or chains of 6, 4 and 2 chromosomes. In this material sporocytes from single anthers show a highly stable pattern of coiling, sporocytes from anthers from different buds of the same plants prepared at different times show different patterns of coiling. The relative stability of coiling is not explained. (B) Observations on coiling in *T. reflexa* strongly favor the two-plane theory of crossing over. Coiling commences during pachytene; average chiasma frequency decreases from 2.6 at early diplotene to 1.8 at MI. In normal *T. reflexa* there are an average of four ring-shaped and two rod-shaped gemini at MI. About half of the gemini contain partners showing opposite direction of coiling at prometaphase. In these, sister chromatids at prometaphase coil the same way. Sister chromatids at anaphase coil oppositely in parts of their course in 14.8 per cent. of dyads derived from rod-shaped gemini and in 24.4 per cent. of dyads derived from ring-shaped gemini. The majority of cases involving fully terminalized rod-shaped gemini with homologous partners coiling oppositely showed sister chromatids coiling in parallel without reversal of direction into the very tips of the chromosomes. On the basis of the one-plane theory one would expect a much larger number of these cases to show reversal of direction of coiling in one chromatid. On the other hand, the observations fit the two-plane theory. The rare cases of change of direction of coiling in parts of sister chromatids in rod-shaped gemini at AI are the non-cross-overs, *i.e.*, terminalized, unbroken chiasmata in the classical sense. The majority of cases, rod-shaped gemini with parallel sister chromatids at AI coiling oppositely throughout homologues, are the result of crossing over, *i.e.*, breakage of chiasmata in the classical sense. Observations on chiasmata show that terminal associations at MI are invariably the result

of subterminal chiasmata of earlier stages which have terminalized.

35. *Genetic Effects Induced in the Sexes:* C. P. OLIVER, University of Minnesota. Young Drosophila females following x-radiation are significantly less fertile than males. Decreased fertility in males corresponds roughly to intensity for the dosages given. High intensity causes a drop greater than expected in the number of offspring per treated female parent. With female treatment, sex-linked lethals and translocations involving X are induced one third to one half as frequently for any intensity as with male treatment, but with either sex treated the ratio of induced lethals to translocations is the same. Crossing-over irregularities are induced less frequently in the female than in the male lines. The rate in the male line equals that of the other effects. In the female line it is greater than the lethal or translocation rate. Visible variations occur in equal numbers following an equivalent treatment of either sex. In the male line this rate is about one seventh as frequent as the rate of the other genetic effects. In the female line it is about one half the lethal or translocation rate.

36. *Salivary Gland Chromosomes in Drosophila melanogaster:* T. S. PAINTER, University of Texas. After briefly discussing the peculiar conditions found in the salivary glands a summary will be given of the results which have been obtained to date at this laboratory through the study of the giant salivary chromosomes. The normal topography of all elements will be shown together with the morphological positions or limits of all genes which have been mapped. Aside from gene localization, which will be discussed, outstanding features are: (a) the presence of inert material in both chromosome II and III, (b) the extension of the arms of the large autosomes beyond any known genes, (c) the relative frequency with which chromosomes "fuse" or form new associations in the region of the chromocenter. The theoretical bearings of these findings will be discussed, as time allows.

37. *A New Aleurone Color in Maize:* MARCUS M. RHOADES, Department of Plant Breeding, Cornell University. A selfed ear of an inbred strain of Black Mexican sweet corn segregated for aleurone color into 12 purple: 3 dotted: 1 colorless seeds. The unexpected dotted aleurone class was caused by the interaction of a new dominant factor (Dt) with recessive a_1. Seeds

of a_1 A_2 C R Dt constitution have dotted aleurone, while seeds of a_1 A_2 C R dt have colorless aleurone. Since Black Mexican strains are not known to carry Dt and are homozygous for aleurone color it seems probable that the mutations of A_1 to a_1 and of dt to Dt occurred in the same cell. Dt is specific in its interaction with a_1, since it produces no color with a_2, c or r. Dt shows no linkage with a_1, a_2, c, r and su. Seeds of a_1^p A_2 C R dt constitution have pale purple aleurone color, and a_1 a_1^p A_2 C R Dt seeds have a pale purple aleurone on which appear the more intense dots of color. The ratio of the number of dots on seeds of a_1 a_1 a_1^p A_2 C R Dt Dt dt genotype to the number of dots on seeds of a_1 a_1 a_1 A_2 C R Dt Dt dt constitution is 2:3, while the ratio for seeds at a_1 a_1^p a_1^p A_2 C R Dt dt dt to seeds of a_1 a_1 a_1 A_2 C R Dt dt dt constitution is 1:3.8. These ratios suggest that the dosage of a_1 affects the number of dots or else that a_1^p has an inhibitory effect which is proportional to the dosage of a_1^p.

38. *The Genetics of Self-sterility in the Genus Capsella:* HERBERT PARKES RILEY, Newcomb College, Tulane University, New Orleans, La., and Bussey Institution, Harvard University, Jamaica Plain, Mass. While self-sterility in most plants is determined by oppositional factors, a few cases have been found which can not be explained in this manner. In *Capsella grandiflora,* the sporophytic constitution of any two plants determines whether they will be sterile together, irrespective of the genes in the pollen grains of the plant used as a male. Two pairs of factors (*Tt* and $S^c s$) determine intra-sterile, inter-fertile classes. Plants bearing the gene *T* will be of the same class. In the absence of *T,* plants with the genes S^c will be sterile together, and in the absence of both, *ttss* plants will be cross-sterile. *T* is dominant to *t* and epistatic to S^c and *s*. The class behavior among self-sterile plants is similar to that in *Lythrum salicaria,* where the genes which determine the long-, mid- and short-styled conditions also determine self-sterility classes. In *C. grandiflora,* the gene *T* must always exist in heterozygous condition, as a plant with that gene will be fertile only with *tt* plants, but S^c may be either homozygous or heterozygous, for $TtS^c s$ plants will cross with $ttS^c s$ plants because of the epistatic condition of *T*. Three self-fertile species of *Capsella* cross readily with *C. grandiflora.* Self-fertility is dominant and seems due to a gene S' which is dominant to S^c and s and forms with them

a series of triple alleles. S' is epistatic to T. Self-sterility is due to a failure of the pollen to germinate in incompatible crosses.

39. *The Place of Genetics in Ten Medical Text-books Now Used at Syracuse University:* ROBERT CUMMING ROBB, Syracuse, N. Y. In view of the fact that none of the authors (of ten outstanding text-books now used at the Syracuse College of Medicine) has ever attended a course in genetics, their modern writings are remarkably sound. Ripe in experience, having seen the fall of many theories, they are still open-minded about sex-determination by chromosomes; Cunningham, Gray and Homans are noncommittal on the etiology of congenital anomalies. Moreover, each new edition shows marked advances. The ninth edition of Holt's "Pediatrics" (1926) stated that the disease most frequently transmitted by heredity was syphilis, but the tenth edition contains a flawless discussion of sex-linkage in hemophilia. In 1929 De Lee's "Obstetrics" showed unfamiliarity with the X-Y chromosomes in man, but now he and Williams each recognizes germinal defects as frequent causes of abortion. W. G. MacCallum now condemns the expression "hereditary syphilis" and describes sickle-cell anemia as a "Mendelian dominant." In general, the medical author does not give due credit to (1) recessives. Thus, Wechsler ("Neurology," 1933) remarks that a given disorder is "a chronic, progressive, fatal disease, usually familial, but neither congenital or hereditary." Likewise, Ordway, in Cecil's "Medicine," notes that "leukemia is familial, but heredity is not a factor in its etiology." (2) Lethals and sublethals are not so diagnosed, although responsible for 13 per cent. of infant deaths in 1929, in contrast to 1.5 per cent. in 1889. (3) The logic of genetics is misconceived. "Slye has analyzed hordes of mice over countless generations, but her results do not prove any hereditary origin, though they do show that the supposed cancer inheritance follows strictly the laws of Mendel" (C. J. Miller, "Introduction to Gynecology," 1931).

40. *Demonstration of Effects of X-rays on Male Germ Cells in Apotettix eurycephalus:* W. R. B. ROBERTSON, Department of Anatomy, State University of Iowa. Newly adult or fourth instar males were given 5,000r and later fixed in Bouin's fluid, part of them 2 hours, part 24 hours, part 72 hours and part 288 hours after irradiation. Each follicle of testis shows in the

series of cysts from its blind end to that connected with the *vas deferens* a seriation of stages of cells from spermatogonia to spermatozoa. All the cells in a cyst are at the same stage of rest or division. Cells with nuclei at some stage near division, from late prophase to near telophase, are most sensitive to the effects of raying. This applies to spermatogonia as well as to spermatocytes, and the parts of the cell affected are the chromosome. At 5,000r no cells in a cyst seem to escape and all seem to be affected to about the same extent. X-raying renders the chromosomes sticky and also causes them to be drawn together if they lie at all near each other. Daughter-chromosomes in disjunction are retarded or blocked in their separation. If late metaphase or early anaphase is hit there result various forms of tetra-polar spindles and finally four-nucleated cells, with nuclei equal or unequal. The most interesting results are shown in the twelve-day-old material where the history of what had happened at irradiation may be traced, in four-headed spermatids, cysts of giant spermatozoa or of varying sized or fragmented sperm heads, cysts of degenerating cells, and the like. Also there are some cases in which there appear cysts of apparently rejuvenated (?) cells, the nature of which is not understood.

41. *New Experiences with Oenothera mut. pollicata:* GEORGE H. SHULL, Princeton University. The most characteristic feature of *Oenothera* mut. *pollicata* is the transformation of the hypanthium of the flower from a hollow tube, traversed by the style, to a solid pedicel intervening between the ovary and the proximal end of the style. Two individuals of this new mutant type were first noted in a pedigree culture in 1932, and reported last year. There have appeared quite independently during the past summer new *pollicata* plants in eight families not directly derived from *pollicata* parents. A consideration of these unexpected new *pollicata* mutants shows that they occur in two unconnected pedigree lines, but that of the eight families in which they have appeared, seven diverge from a common pair of ancestors grown in 1928. It appears certain, therefore, that the *pollicata* gene-mutation in these lines appeared at least as far back as this common ancestral pair and has been carried along hidden in this sector of my pedigree cultures because of its recessive nature and its linkage with one of the *Lamarckiana* zygote lethals. That *pollicata* is definitely a recessive of the first linkage group is decisively proven by the fact that in three of the

eight families in which it has newly appeared, the parent was of the *erythrina* type, and the progenies have shown the usual segregation of *erythrina* and *decipiens* in a 2:1 ratio; in these three families all the *decipiens* plants have been *pollicata,* while only one of the *erythrina* plants was a *pollicata,* this last produced by a transfer of the *pollicata* gene from the haplo-*decipiens* complex to the *velans* complex.

42. *Extra-genic Effects in Offspring Arising from X-rayed Gametes in Drosophila melanogaster:* E. LUCILE SMITH, Departments of Anatomy and Zoology, State University of Iowa. Increased appearance and degree of irregularity of irregular abdominal sclerites occurred in both male and female offspring of females that prior to mating had been treated with X-rays. The character consisted in that entire segments might be missing, or right, left or median dorsal half of segments. In some cases this produced peculiarly rotated abdomens. Twenty-nine control females (Group I) produced 3,023 F_1s, of which 29 (.96 per cent.) had abnormal abdomens; twenty-nine females (Group II), irradiated with 731r, gave 2,997 F_1s, of which 35 (1.17 per cent.) had abnormal abdomens; twenty-four females (Group III), irradiated with 1,462r, gave 2,638 F_1s, with 82 (3.11 per cent.) that had abnormal abdomens. Among the irregular offspring of controls the irregularity affected only one segment in 80 per cent. of the individuals and produced no rotated abdomens; of Group II, 74.2 per cent. had one segment affected, but 25.8 per cent. had two or more segments irregular or had rotated abdomens; of Group III, 72.1 per cent. had one segment irregular and 27.9 per cent. had two or more segments irregular with rotated abdomens. It seems that the irregularity is greater with increase of irradiation. As to the inheritance of the character it was found that the F_1, F_2 and F_3 generations from parents with abnormal abdomens, derived from Groups I, II and III, had essentially the same per cent. of irregularities as the F_1 generation of the original control females. The change seems therefore not to be inherited, in this case, and it would appear that x-rays can affect the cytoplasm, or non-genic portion, of the gamete in such a way that the individual developing from it shows the change.

43. *Chromosome Fragmentation Produced by Crossing-over in Trillium erectum L.:* STANLEY G. SMITH, Department of Genet-

ics, McGill University. Chromatid fragmentation and the production of chromatids with two attachments are shown to result from chiasma formation in an inverted segment.

44. *The Application of the Gene-frequency Method of Pedigree Analysis to Sex-influenced Factors:* LAURENCE H. SNYDER, Ohio State University. The gene-frequency method of analyzing human pedigrees is extended to include sex-influenced factors by making use of the equation $\sqrt{\overline{♀B}} + \sqrt{\overline{♂b}} = 1.0$, where $\overline{♀B}$ represents the proportion among females of females who show the character represented by B, and $\overline{♂b}$ represents the proportion among males of males who show the character represented by b. The probable error for the deviation is derived and presented. The equation is used to test the sex-influenced nature of the human character baldness. The proportion of baldness in women was accurately obtained by a study of 3,966 inmates of state hospitals for the insane. The deviation from unity is $.03 \pm .009$. Family histories involving baldness are presented, indicating that the sex-influenced basis for the inheritance of the character is the most reasonable explanation.

45. *Cell Size and Chromosomal Types in Habrobracon:* B. R. SPEICHER, Carnegie Institution of Washington, Cold Spring Harbor, N. Y. Each cell of the single layer forming the upper surface of the primary wing bears a single bristle, so that variations in cell size (surface area) produce proportional variations in dispersal of bristles. By counting bristles within a set area (0.032 sq. mm in R_2 wing cell) from sample populations an approximation of cell size may be obtained. This was done for 83 haploid males, 52 diploid males and 40 diploid females derived from inbred stock 11 and its closely related stock 11-o, and also for 83 haploid males and 85 diploid females from inbred stock 25. Ten triploid females were also studied, but the significance of their measurements is doubtful since they were not inbred and probably carried the factor shot-veins, affecting the wings. Triploid females have largest cells, 1056.3 ± 59.5, measured in sq. micra $\pm$ S.E. In stocks 11 and 11-o combined, cell size is in the following decreasing order: diploid males 690.1 ± 8.5, diploid females 536.9 ± 5.1 and haploid males 474.7 ± 2.0. In stock 25 cell size is larger but in the same order: diploid females 600.0 ± 9.6, haploid males 568.1 ± 4.3. Total wing area, measured in sq. mm $\pm$ S.E., may differ with sex (stocks 11 and 11-o

diploid females 0.9045 ± 0.015, haploid males 0.8243 ± 0.013, diploid males 0.7761 ± 0.013) or may be similar (stock 25 diploid females 0.7410 ± 0.007, haploid males 0.7428 ± 0.009). Triploid females possess smallest wings, 0.7134 ± 0.016.

46. *Phenogenetic Peculiarities in Drosophila funebris:* WARREN P. SPENCER, College of Wooster. Among 46 natural visible mutations found by the author in 10 species of Drosophila other than funebris 13 or 28 per cent. have been color mutants, 23 or 50 per cent. have shown 100 per cent. phenotypic manifestation (penetrance of Timofeeff-Ressovsky) under standard laboratory conditions, and 11 or 24 per cent. have been primarily bristle mutants. In contrast, of 20 natural visibles among *D. funebris* none have been color mutants, only 3 or 15 per cent. have given 100 per cent. manifestation, while 11 or 55 per cent. have been bristle mutants. These findings agree with those of the European workers on this species both in respect to natural and x-ray mutants. Other peculiarities include a high per cent. of dominants, reversed dominance relations in various stocks, and extremely high variability in phenotypic expression even in stocks inbred for years.

47. *Genetic Behavior of a Haplo-viable Internal Deficiency in Maize:* L. J. STADLER, U. S. Department of Agriculture and University of Missouri. This deficiency, designated Df 5_1, was induced by x-ray treatment of mature pollen. It involves a short internal segment of the longer arm of chromosome 5. Plants heterozygous for the deficiency are equal to their non-deficient sibs in growth and are distinguishable only by the defective development of half of their pollen. The deficient pollen grains are reduced in size and incompletely filled. They rarely germinate, and the deficiency is not transmitted through the pollen. It is regularly transmitted through the female gametophyte. Heterozygous deficient seeds are slightly but consistently smaller than adjacent non-deficient seeds. The deficiency includes the locus of the gene V_3, but not the neighboring loci of Bm_1, *Bt* or *Bv*. At pachytene in heterozygous plants the non-deficient chromosome shows a short-buckled region, usually on the longer arm near the spindle node. This position varies, and in extreme instances the buckle may occur in the distal half of the longer arm or in the proximal region of the shorter arm. When the buckle is on the shorter arm the spindle nodes of the paired

chromosomes are separated by a distance approximately equal to the length of the buckled region. When the position of the buckle is not identical with the locus of the deficiency, the chromosome regions paired between these two points must be non-homologous. By crossing over within this region progeny bearing the gene V_3 and a deficiency in the same chromosome might be secured. Such plants are found. Crossing over is greatly reduced in frequency in heterozygous deficient plants, not only in the *Bm-Bv* region, which includes the deficiency, but also in the adjoining *Bv-Pr* region.

48. *Chromosome Structure and the Mechanism of Meiosis in Plants:* G. LEDYARD STEBBINS, JR., Colgate University. In the root tip cells and microsporocytes of *Paeonia,* each chromosome consists, except during the anaphase, of four chromonemata surrounded by a matrix. During the chromosome cycle these chromonemata vary in structure and relative position. They split at the late anaphase or the early telophase. The chromonemata of different chromosomes remain distinct from each other throughout the telophase, and probably the resting stage. At the telophase of the last premeiotic mitosis, each chromosome contains likewise four chromonemata, so that the earliest prophase of both mitosis and meiosis, as observed in *Paeonia, Allium* and *Tulipa,* consists of the coming together of four chromonemata, accompanied by their elongation. In mitosis, optically double spireme threads are formed, while in meiosis the four chromonemata are almost completely fused, as chromosome pairing begins. The initial differences between mitosis and meiosis are the greater length of the meiotic prophase and the closer approximation of the chromonemata of each chromosome, followed by chromosome pairing. A hypothesis to explain these differences is that the physiological condition of the nucleus which, in the early prophase of every mitosis, promotes the coming together of chromonemata, is prolonged in the meiotic prophase, so that homologous as well as sister chromonemata come together. Evidence from hybrid and other asynaptic strains tends to support this hypothesis, and indicates that the physiological condition mentioned is connected with the growth and nutrition of the cell and nucleus.

49. *Further Studies on Somatic Crossing-over and Segregation:* CURT STERN, University of Rochester. The studies on

somatic crossing-over and segregation mainly of the X-chromosomes in *Drosophila melanogaster* have been continued with special reference to the question of the sex of the mosaic spots. While the earlier work of Bridges on supposed chromosomal elimination seemed to contain evidence for the male constitution (1X) only of the spots on females, the work of the writer demonstrated the occurrence of spots of both sexes. It could be shown that the appearance of female spots is the rule in cases where the genes of the two X-chromosomes are present without duplications. However, the presence of a duplication for these genes or of a Y-chromosome can lead to types of somatic crossing-over and segregation which cause the appearance of spots of different sexes. An attempt will be made to explain the behavior of "mottling" and of "unstable" translocations and duplications on the basis of somatic crossing-over and segregation in females and males.

50. *A New Linkage Group in the Fowl (Gallus domesticus):* D. C. WARREN, Kansas State College. There are known in the domestic fowl, in addition to the sex-linked group, two autosomal linkage groups. Rose comb and creeper constitute the first known group, and these two factors have been shown to be very closely associated. The factors dominant white, crest and frizzling have been demonstrated to belong to another group. Recent work by the writer has also shown that silky plumage and flightless constitute a third group of linked autosomal characters. Silky is a recessive variation in feather structure (lack of hooklets) and flightless is a dominant defect in feather formation causing the larger feathers of the wing and tail to break off and render the bird flightless. A study of linkage in the repulsion phase gave 219 non-crossovers and 22 crossovers in a test for crossing over in the female and 127 non-crossovers to 16 crossovers when the test was in the male. Calculations from these data give 11 per cent. crossing over in the female and 9 per cent. in the male. These results are in agreement with the earlier obtained evidence indicating that in the fowl the percentage of crossing over is higher in the female than in the male.

51. *Impaternate Daughters of Females Heterozygous for a Sex-linked Gene in Habrobracon:* P. W. WHITING and KATHRYN GILMORE SPEICHER, Carnegie Institution of Washington, Cold Spring Harbor, N. Y., and Pennsylvania College for Women.

Impaternate females have approximated 1AA:2Aa:1aa for genes for which their mothers were heterzygous, Aa. However, since fused, fu, is sex-linked, impaternate daughters from Fu/fu mothers should be heterozygous except for crossovers between XY and Fufu. 95 impaternate daughters from such mothers proved to be,—wild-type 25 untested Fu/?, 64 Fu/fu, 2 Fu/Fu, and fused 4 fu/fu. It is probable that many homozygous, Fu/Fu or fu/fu, impaternate offspring occurred, but that the majority of these were diploid males, XX or YY, and non-separable from their haploid brothers, X or Y.

52. *Analysis of Growth Rates of Genetically Different Strains of Daphnia longispina:* THELMA R. WOOD and A. M. BANTA, Brown University and Department of Genetics, Carnegie Institution of Washington. Of 185 *Daphnia longispina* females from two slow-developing dwarf and four normal clones (3 related and 1 unrelated) reared simultaneously (25° C.) from birth until death, 149 were cultured under optimum food conditions, whereas 18 of each of the two dwarf lines were reared in diluted medium (starved). Early growth increments of slow-developing dwarfs are much smaller and duration of early instars, particularly the second, is much greater than among the normal clones. Adult percentage growth increments of dwarfs are larger than those of the normals. Dwarfs produce their maximum broods two or more instars later in life than the normals. Dwarfs live longer than normals. Dwarfs starved show even greater retardation in development, reduction in numbers of offspring and increased longevity. Comparison of these facts with the known developmental and reproductive performances of the unrelated normal clone when reared (1) under optimum conditions (2) starvation conditions and (3) starvation conditions to maturity (followed by optimum food conditions), suggests that in our cultures the slow-developing dwarf clones are really starved during early development and have more optimum conditions during later development. Previously reported breeding experiments indicate that this factor for slow-developing dwarfism is recessive. It seems that this gene may be responsible either for the tardy development of cellular elements or enzymes necessary for normal assimilation of food substances or for a need by the animals of some substance present only in small amounts in the food serving as optimum for most Cladocera lines.

SHORTER ARTICLES AND DISCUSSION

THE USE OF DOUBLE MONSTERS AS MEANS OF IDENTIFICATION IN CROSSING PURE LINES OF PARAMECIUM CAUDATUM

DURING a study of axiate organization in the ciliates, it was found that cell division in paramecium could be inhibited by reduction of temperature or by cyanide vapor in such a way as to produce double monsters. Typically these monsters are at first L-shaped, the axes of anterior and posterior components intersecting to enclose an angle of about 90 degrees. At the intersection of their axes the two components are in free cytoplasmic continuity, there being an irregular streaming or cyclosis from one component to the other. Usually on the second or third day after monster formation (sometimes at a longer interval) one or both components undergo division, resulting in free individuals which are then available for continuing pure lines. The residual monster may again divide, or partially divide, several times in one or both components, or in new axial protrusions, over a period of three to thirteen days. But all such monsters eventually end their existence as complex branching masses without apparent axiate organization.

With the purpose of finding out whether or not the experience of monster formation alters the genetic constitution of paramecium, twenty-eight pure lines of *P. caudatum* were studied as to fission rate and cell length over a period of eighty-five days. Then in each line experimental monsters were produced by cyanide vapor or by reduction of temperature; and free individuals from each monster were used to start new pure lines, which in turn were studied again as to fission rates and cell lengths for a period of eighty-five days. The results of this work clearly demonstrated that as to the measurable characters, fission rate and cell length, the genotypes are not changed by the experience of induced monster formation (De Garis, 1927).

During 1928 further work with paramecium monsters happened to reveal the fact that such monsters on occasion evince the faculty of conjugating with free individuals, either one or both components being so engaged. The first observed conjugations were between double monsters and free individuals of the same clone. The question arose: Would monsters of any line conjugate with free members of any other line, provided only

one or the other line were ready for conjugation? In other words, is there anything in the condition of monster formation alone which conduces to conjugation? Repeated experiments answered this question in the negative. The monsters and free individuals, in order to effect conjugation, have to be derived from lines which are themselves ready for conjugation. The fact, however, that conjugation could be effected between double monsters and free individuals suggested the possibility of using the double monster condition as a definite means of identifying or, as it were, tagging members of one line in crossing with another line.

The double-monster condition, while especially useful as a means of identifying diverse members of a given cross, has also the advantage of representing a retarded physiological state, apparently a suspension of nuclear changes, such that a monster in a conjugating line will still take part in conjugation for two or three days after all free individuals of the same line have ceased to mate. As far as the present work goes, nothing has been learned regarding these possible nuclear changes. Yet the prolongation of propensity to conjugate has been demonstrated with such regularity that it must be considered a definite consequence of the monster condition, and one of great usefulness in effecting crosses between lines which conjugate only a few days apart.

A further advantage in the use of double monsters for conjugation purposes arises from the fact that by this means the difficulty of assortative mating may be avoided, and long may be mated with short, provided the monster is produced in the long line. Otherwise such mating can not be accomplished, or at least has not been in the course of the present work. However, the fact that long can be crossed with short is not only of interest in itself, but greatly extends the application of this method to the crossing of diverse lines.

One of the difficulties to be encountered in using double monsters as conjugants arises from the fact that the monster components may become separated during, or shortly after, the mating process. If this separation occurs after the monster with mates has been isolated from the mass conjugating culture, then the identification of anterior and posterior pairs is usually rendered possible by a truncated or notched condition at the apical or caudal extremity of respective monster components, indicat-

ing the site of their former union. But if the separation occurs while the conjugant group (monster and mates) is still in mass culture, the identification of separated pairs may well be impossible.

Experience shows that the separation of monster components usually does not take place if there is free cytoplasmic communication between components—a condition which may be assured if the cells chosen for monster formation are taken at the very beginning of division. As a practical matter, the cells most suitable for effective monster formation are those with marked equatorial thickening and with new contractile vacuoles already present. If cell division has advanced to the stage of deep constriction before cyanide vapor is applied, a monster may and usually is produced. But its components will be united by nothing more than a bridge of ectoplasm, and will almost certainly be broken apart during or after conjugation.

Another occasional difficulty in the use of monster conjugants is due to the fact that some monsters, whether ex-conjugant or non-conjugant, give off fragmentary or distorted daughter-cells. These may become adjusted through subsequent divisions to perfectly normal form, or they may fail to undergo further division. Thus far no way has been found to circumvent this difficulty. Fortunately its occurrence is quite the exception rather than the rule.

The method described above for the use of double monsters in effecting crosses between pure lines of paramecia has been successfully used by the author for a number of years. Some of the genetic results in crossing pure lines of *Paramecium caudatum* by this method have already been announced (De Garis, 1930, 1934). The same method has been extended to crossings of *P. caudatum* with *P. aurelia* (results unpublished).

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LETHAL EFFECTS OF CONJUGATION BETWEEN PARAMECIUM AURELIA AND DOUBLE MONSTERS OF P. CAUDATUM

THE problem of effecting crosses of the Protista, whether between diverse lines of the same species or between different species, has not until recently met with much success (except for the decisive work of Pascher (1916, 1918) on Chlamydomonas). The conditions leading to conjugation and the results arising from it have been considered in much detail by Jennings (1910, 1911, 1913), and the attempts at race- and species-crossings have been reviewed by Jennings (1929) and by Sonneborn and Lynch (1934).

Systematic work directed to the study of the genetic consequences following conjugation between diverse races of *Paramecium caudatum* was begun by the author in 1928, and the results were very briefly announced two years later (De Garis, 1930). Results from an extension of this work were announced, again briefly in 1934. Meanwhile the work of Müller (1932) on the crossing of *Paramecium multimicronucleatum* and *P. caudatum* promises much light on the cytological changes attending such crosses. Finally the experiments of Sonneborn and Lynch (1934) solve the difficult problem of race crossing in *Paramecium aurelia.*

The present report sets forth the results of crossing pure lines of *Paramecium aurelia* with *P. caudatum* by the use of double monsters experimentally induced in the latter species. The propositions on which this work is based may be enumerated as follows:

(1) *Paramecium caudatum,* taken at the beginning of cell division, may be so depressed by reduction of temperature or by cyanide vapor as to carry out only the nuclear changes, the two daughter cells remaining in cytoplasmic union and thus forming the components of an L-shaped double monster.

(2) Such a monster may, and usually does, subsequently divide in anterior or posterior component, or both, to produce free and apparently normal individuals.

(3) These individuals may be used to start pure lines, and these lines are found to have the same genetic constitution (as measured by fission rate and cell length) that characterized the pure lines before monster formation. In other words, it has

been shown that the genotype is not altered by the experience of induced monster formation (De Garis, 1927).

(4) It has further been observed that induced monsters of one race will conjugate with free individuals of another race, provided both races are ready for conjugation. Thus the monster condition offers a definite and simple means of identifying the participants in a given cross. The procedure is briefly this: A double monster is produced in one line, *e.g.*, by cyanide vapor, and this monster is introduced into a conjugating population of the other line, where it will soon acquire mates, either for one or both its components. When the monster and its mates are fully engaged in conjugation, they may be isolated in a slide depression, and the process of conjugation observed to completion, with the separation of known individuals, and the starting of new lines from these individuals. This method and some of the genetic results have already been announced (De Garis, 1930, 1934). Similar results by a different method are reported by Sonneborn and Lynch (1934) for *P. aurelia.*

In the course of this work with monster crosses it was found that the difficulty of assortative mating, studied by Jennings (1911), is avoided, and short mated with long, provided the monster is produced in the long line. Otherwise such mating can not be accomplished, or at least has not been during the present work.

With the foregoing facts in mind, it was considered feasible to attempt crossings between *Paramecium aurelia* and *P. caudatum* by inducing monster formation in the caudate line and introducing this monster into a conjugating population of *P. aurelia.* An abundant supply of *P. aurelia* was kindly furnished me by Dr. J. W. Buchanan, of New Haven.

This work of crossing the two species of paramecium was done during the summer of 1930. In preparation, much effort was expended in establishing and caring for the pure lines and their parallel mass cultures, daily records being made of fission rates and cell lengths in thirty-four lines of *P. aurelia* and thirty-seven lines of *P. caudatum,* together with conjugation records of an equal number of watch-glass mass cultures for each species. The actual experimental work of crossing the lines of these species, however, did not extend beyond preliminary tests, the results of which were as follows: The caudate monsters, introduced into conjugating populations of *P. aurelia,* readily acquired mates of the latter species, and within an hour or two

conjugation was well under way, so that monsters with mates could be isolated. The mates remained adherent to the monsters for a number of hours (five to twelve). In four instances, they did not become disengaged at all, but eventually underwent cytolysis (during the second day) and were dragged about by the monster as so many flaccid bags. The free mates that did become disengaged invariably died on the second or third day after conjugation. Characteristic changes indicating the approach of death were: excessive swelling of the organisms, clouding of the cytoplasm with eventual accumulation of large vacuoles throughout, contractile vacuoles not visible, ciliary movement increasingly slow to the point of complete cessation. The exact moment of death could only be fixed as that at which the cell membrane ruptured and the vacuole-laden cytoplasm was extruded. The foregoing degenerative changes are, in a general way, comparable to the process in mammalian pathology known as "cloudy swelling." It should be recalled that all the free mates, whose manner of death has just been described, were *P. aurelia.*

The monsters, all of which were *P. caudatum,* lived for an average period of six days after conjugation. Of these monsters, numbering seventeen in all and representing three diverse lines, two died on the second day after conjugation, two on the third day, three on the fourth, four on the sixth, one on the seventh, three on the eighth, one on the eleventh and one on the twelfth day. This record of duration is fairly comparable to that of monsters which have not conjugated. A striking difference, however, resides in the fact that none of these conjugant monsters gave off any free cells after conjugation. Indeed, there was not the least sign of growth in any of the monsters, some even appearing smaller than when first formed. Nor was there any appreciable change in configuration. Instead of the bizarre, branching monsters, residual from various divisions and protrusions, these monsters remained L-shaped throughout their lives.

Some hours before death each of these monsters underwent certain characteristic changes. The cytoplasm became hyaline in appearance and free of granules and vacuoles, the contractile vacuoles ceased to function and became greatly distended, ciliary movement gradually slowed and terminated in a tail-to-head wave, the apical cilia being the last to move. A few seconds thereafter one or another retention cyst of a contractile vacuole ruptured and thus that particular component of the monster

perished. The other component went through the same stages of disintegration, either simultaneously or shortly afterward. The foregoing changes may, in a general way, be referred to as "hyaline degeneration."

The results of these species crosses can not be fully interpreted without cytological examination to ascertain the nuclear distribution. Staining methods adequate to this purpose will be undertaken during the present summer (1934). In the absence of this information regarding nuclear distribution, certain conclusions are still permissible.

SUMMARY AND CONCLUSIONS

Crosses between the two species, *Paramecium aurelia* and *P. caudatum*, have been effected by means of the double monster condition induced in the caudate lines. Thus the difficulty of assortative mating is avoided, and short mated to long, when the monster condition is induced in the long lines. Such crosses are invariably lethal to *P. aurelia*, death ensuing within two to three days by a degenerative process comparable to "cloudy swelling." *P. caudatum* in monster condition is completely inhibited as to growth and cell division by the experience of conjugation, and after a period of two to twelve days ends its existence by a pathological process similar to "hyaline degeneration." The results of these crosses are distinctly not encouraging to the view that these two species can be crossed to produce some new form of animal life.

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THE INHIBITION OF REGENERATION BY X-RAYS, IN THE URODELE, *TRITURUS VIRIDESCENS*

VARIOUS investigators[1] have reported inhibition of regeneration by x-rays in invertebrates and larval vertebrates. The general practise has been to irradiate the entire animal, and several workers in this field have reported lethal action for those dosages which were sufficiently strong to inhibit regeneration. In the present study, only adult vertebrates were used and these were protected by a quarter-inch lead shield and only the desired portion of the body was exposed. As a result of this precaution, the mortality of the experimental animals was no higher than that of the controls.

To cite a typical experiment, the average regeneration of the tails of the irradiated specimens was less than one millimeter in 105 days. The tails of the non-irradiated controls, collected and maintained under identical conditions, showed an average regeneration of over nine millimeters in the same period. Over 150 animals have been used in this series of experiments, with marked inhibition in every case. There is, as is usual with live biological material, a great deal of variation in individual susceptibility. The length of the regenerated tail in the irradiated individuals, however, was in no case greater than 3.4 millimeters, and in a

[1] C. R. Bardeen and F. H. Baetjer, *Jour. Exp. Zool.*, I: 191, 1904; W. C. Curtis and Jane Hickman, *Anat. Rec.*, 34: 145, 1926; W. C. Curtis, *Science*, 67: 141, 1928; R. G. Stone, *Jour. of Morph.*, 53: 2, 1932; G. Bolitzer, *Roux Archiv*, 121 (1/2): 39, 1930; E. G. Butler, *Science*, 74 (1908): 100–101, 1931.

few cases there was a slight shrinkage instead of growth. This was probably due to voluntary fasting. The non-irradiated newts, in contrast to the others, showed regeneration of from 6 to 17½ millimeters.

Godlewski[2] found that regenerative processes were inhibited by covering over a wound with living epidermis from another part of the body of the same animal. This suggested that what appeared to be inhibition of regeneration by x-rays might possibly be an indirect effect brought about by an abnormal stimulation of wound healing. In order to test this view the tails were amputated a second time, 105 days after the date of the original operation and irradiation, and about one or two millimeters craniad of the plane of the first amputation. After 33 days of growth, it was found that the average length of the regenerating tails was less than one millimeter in the experimental group, whereas in the control group it was over three millimeters, thus showing that the inhibition was brought about directly by irradiation.

The animals used in these experiments were exposed to a dosage of 1704 "r" units during a period of twenty minutes. Reducing the length of the exposure to five minutes, 446 "r" units, still gives a dosage sufficient to inhibit regeneration. Sixty-eight days after this reduced exposure the average regeneration for the experimental series was zero, while that for the control series was slightly less than five millimeters. The results

[2] Emil Godlewski, Jr., *Roux Archiv*, 144 (1): 108–145, 1928.

EXPLANATION OF FIGURES

1. Photograph showing complete inhibition of the regeneration of the tail for 295 days after exposure to x-rays. Above is a non-irradiated control animal showing normal regeneration for 307 days. Below is an irradiated individual. The tails of both were amputated on the same day. Twelve days after amputation the experimental animals were exposed for twenty minutes to a dosage of 93.75 roentgen units per minute.

2. Photograph showing the abnormal growth subsequent to an exposure to x-rays which was made sixty days after amputation. On the extreme left is shown a control individual. The dosage used in this series was the same as that given above. Growth before irradiation was normal in all respects.

3. Photograph showing the abnormal growth subsequent to a five minute exposure to a dosage of the same strength as that given in the two series mentioned above. In this series the x-ray exposure was given on the same day as the tail was amputated. Sixty-eight days after irradiation, inhibition of regeneration was apparently complete; however, within the next eighty days there was a slight but abnormal growth. The individual on the extreme left of the photograph is a non-irradiated control.

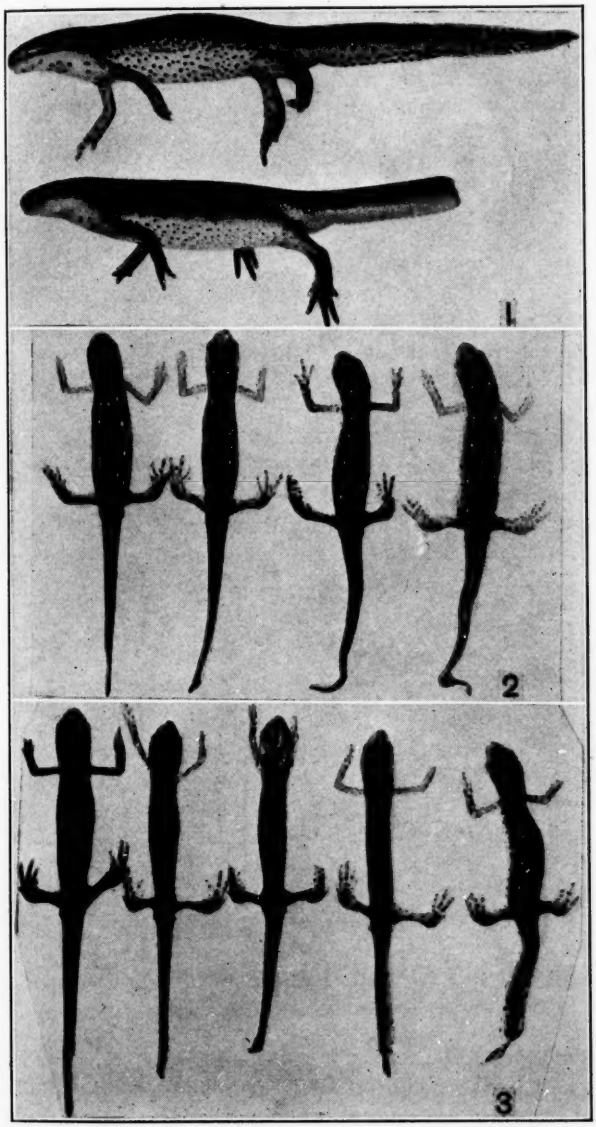

PLATE I

of these experiments show quite definitely that x-rays inhibit regeneration in adult Triturus as well as in larval vertebrates and adult invertebrates.

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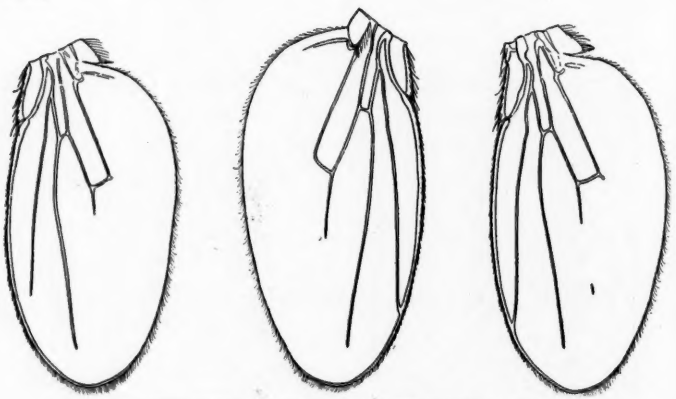
VEINLET, A FIRST RANK RECESSIVE, LOCATED JUST TO THE RIGHT OF ROUGHOID IN CHROMOSOME THREE OF *DROSOPHILA MELANOGASTER*

OF the few mutants previously known to be located near the extreme left end of the third chromosome, only one, *viz.*, roughoid, can be used well with other characters; therefore the appearance in this region of another mutant with well-defined characters is of some importance. It is probable that this mutant will displace roughoid in usage, since its classification is certain and rapid, while roughoid is slow in classification and sometimes is so slight in degree as to overlap the wild type phenotypically. Also the new character affects the veins instead of the eye surface and hence can be used with the essential character rough, while roughoid and rough could not be used together successfully.



The mutant veinlet, showing the small range of variation.

The mutant, veinlet, was found (January, 1933) in a stock culture of yellow[2]. The third, fourth and fifth longitudinal veins of this mutant do not extend to the marginal vein. The fourth and fifth veins extend only a short distance beyond the posterior cross vein. It is an easily recognized character and

can be used with any other character except those that materially affect the wing. In the double recessive veinlet hairy, the hairs extend beyond the incomplete veins, but are not dense enough to make classification of veinlet difficult. In the triple recessive ru ve h there is more variation toward normal in all the veins except the fifth, which always remains characteristic for veinlet. The character does not overlap the wild type and in other characteristics of viability, fertility, etc., is of the first rank.

The gene for this character was shown to be located in the third chromosome. A stock was then made having the characters veinlet, ve; thread, th; scarlet, st; sooty, e^s; and claret, ca. This was mated to wild stock and the female offspring backcrossed to ve th st e^s ca males in order to determine the recombination values. There were classified 1,634 flies, with the following results:

0: 181 + 300 = 481;
1: 185 + 133 = 318;
2: 2 + 0 = 2;
3: 38 + 81 = 119;
4: 145 + 231 = 376;
1,3: 51 + 19 = 70;
1,4: 127 + 91 = 218;
2,4: 0 + 1 = 1;
3,4: 0 + 33 = 33;
1,3,4: 15 + 1 = 16.

In the tabulations the class beginning with veinlet is put first and the complimentary class second, *e.g.,* non-crossovers are ve th st e^s ca, 181 and normal 300, equaling 481.

The total of observed crossovers between veinlet and thread was 622, which gives 38.1 per cent. recombination for these genes. The standard map shows that there is 42.2 per cent. crossing-over between roughoid and thread. Since there are few genes known near the left end of the third chromosome, it seemed desirable to locate the gene for veinlet more definitely with reference to roughoid.

Stock was made with the characters veinlet hairy thread and the recombination values determined. In this experiment 5,594 flies were classified. The following tabulation follows the same order as that given above:

0: 1,221 + 1,661 = 2,882;
1: 698 + 570 = 1,268;
2: 628 + 584 = 1,212;
1,2: 73 + 59 = 132.

The total number of observed crossovers between veinlet and hairy was 1,400, giving a recombination per cent. of 25.0. The total observed crossovers between veinlet and thread was 2,744, giving 49.0 per cent. recombinations. The value 25.0 for ve–h is so near to the value 26.5 expected for ru–h that one can not determine from this experiment on which side of roughoid the gene for veinlet lies.

A stock containing the genes for roughoid veinlet hairy thread was made as follows: ve h th was mated to ru. The female offspring were then mated to ve h th males to provide the data given above. From this cross about a hundred of the ve h th offspring were selected, either male or female, and mated to roughoid. If any one of them should have the genetic constitution ru ve h th/ ve h th, this could be recognized in the offspring, half of which would be homozygous for roughoid. These roughoid flies, ru ve h th/ ru, were found in one of the cultures and were mated together. From their offspring was obtained the ru ve h th homozygous stock.

From the data given above veinlet must lie very near to roughoid and it must be to the right of roughoid if the roughoid gene entered the chromosome by a single crossover. A double crossover could occur, but the probability of finding one by the method given above is negligible.

Some of the roughoid flies heterozygous for ve h th were mated to hairy. The female offspring, which were hairy, were then mated to ru ve h th males to determine the percentage of recombination for roughoid and veinlet. There were classified 2,833 flies with results as follows:

ru ve h, 1,369;
h, 1,458;
ru h, 2;
ve h, 4.

The observed crossovers were six, giving a recombination value of 0.21 per cent. for roughoid and veinlet. The locus of the gene for veinlet can therefore be placed at 0.2, since it is known to be to the right of roughoid.

For advice in the preparation of stocks and helpful suggestions throughout the experiment, I am under obligations to Dr. C. B. Bridges.

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